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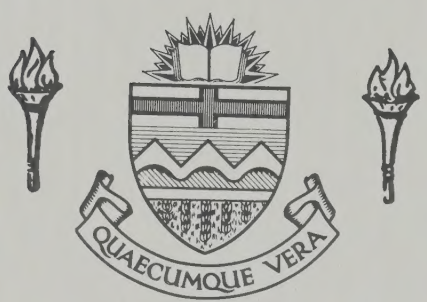

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FACULTY OF GRADUATE STUDIES AND RESEARCH

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "Evidence for a Parathyroid-Dependent Circulating Hypertensive Factor and Its Role in Essential Hypertensive" submitted by Richard Z. Lewanczuk in partial fulfillment for the degree of Doctor of Philosophy.

DATED April 21 1992

To my wife and son,

for their love, understanding and encouragement

ABSTRACT

A number of enigmas exist in the field of hypertension. First, it is known that the parathyroid gland plays a role in some forms of hypertension, yet parathyroid hormone (PTH), its primary product, has consistently been shown to be hypotensive. Second, both calcium and calcium channel blockers have been shown to decrease blood pressure in the same groups of hypertensive patients or animals. Third, although PTH levels are increased in some forms of hypertension, the actions of PTH are seemingly inhibited. To reconcile these observations, it was hypothesized that a circulating inhibitor of PTH might exist in some forms of hypertension, and that such an inhibitor might also be parathyroid gland-dependent. As evidence has long existed for the presence of novel circulating hypertensive factors, and as PTH is hypotensive, it was also hypothesized that a PTH-inhibiting factor might have hypertensive properties.

In this study, dialyzed plasma from Spontaneously Hypertensive Rats (SHR) was found to have hypertensive properties. These hypertensive properties were characterized by a delayed, prolonged increase in blood pressure when injected into normotensive rats. Such plasma also stimulated calcium uptake into vascular smooth muscle and potentiated the actions of other calcium-dependent pressors, suggesting that the factor might act by increasing calcium entry into vascular smooth muscle. This factor did not possess ouabain-like activity and its actions were blocked by calcium channel blockers. The factor did inhibit the vascular actions of PTH and its

secretion was inhibited by a high calcium diet. Parathyroidectomy of SHR rats resulted in a decrease in blood pressure and in the disappearance of the factor. Transplantation of parathyroid glands from SHR to normotensive rats increased the blood pressure of the recipients and resulted in the appearance of the factor in their plasma. On this basis, the factor was named "Parathyroid Hypertensive Factor", or "PHF".

PHF was subsequently shown to be present in a proportion of human essential hypertensive patients -- specifically, in low renin and salt-sensitive patients, who also demonstrate abnormalities in calcium metabolism. In primary hyperparathyroid patients, PHF was found in hypertensive, but not normotensive, patients, and disappeared post-parathyroidectomy. PHF was further shown to be present in low renin (DOCA-salt), but not high renin (two kidney-one clip), rat models of hypertension. Similarly, PHF was present in salt-sensitive (Dahl-S), but not salt-insensitive (Dahl-R), rats.

These findings explain the importance of the parathyroid gland in hypertension and explain why dietary calcium and calcium channel blockers are effective in the same patients. It also explains the association between salt-sensitive, low renin hypertension and abnormal calcium and PTH metabolism. PHF has now been purified and many studies are underway to characterize the substance, both physically and physiologically, and to further understand its role in hypertension.

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INTRODUCTION

Hypertension is a common condition, affecting 20 to 25% of the general population (1). As such, it is a major risk factor for stroke and coronary artery disease (2,3). Contrary to common belief, however, hypertension is not a disease. Rather, it is a physical sign, much as fever or tachycardia are physical signs. As a physical sign, hypertension must have a cause, or causes. Unfortunately, the causes of this condition are generally not known, resulting in most cases of hypertension being classified as "essential" or idiopathic. In recent years, although the cause(s) of hypertension have not been identified, numerous pathophysiological abnormalities have been described in hypertensive humans and animals. The diversity of these abnormalities strongly suggests that there are, indeed, multiple causes for hypertension. For example, under identical metabolic conditions, some hypertensive patients show low plasma renin activity, while other patients show high plasma renin activity (4). Such physiological states cannot, on the basis of current knowledge, be reconciled with a single cause for hypertension. Similarly, in laboratory animals, hypertension can be induced by a variety of interventions. These interventions all induce hypertension, yet the pathophysiological changes they cause in the respective animals differ widely. In the work herein described, one form of hypertension was examined in an effort to gain an insight into its causation. Such a study was prompted by preliminary investigations into the vascular actions of parathyroid hormone in hypertension. During these latter

studies, it became apparent that the existing dogma regarding the roles of calcium and calcium regulatory hormones in hypertension had a number of inconsistencies and unanswered questions. These are detailed below.

Calcium has been presumed to play a role in the genesis or maintenance of hypertension because of its critical role in cardiac and smooth muscle contraction. Because blood pressure is the product of cardiac output and peripheral vascular resistance, anything that affects the latter two processes must also affect blood pressure. Thus, factors affecting cellular calcium metabolism in the heart and vascular smooth muscle would also be expected to affect blood pressure. The first described relationship between hypertension and calcium regulation was in primary hyperparathyroid patients, as described by Hellstrom *et al.* in 1958 (5). In this report, a high prevalence of hypertension was described in primary hyperparathyroid patients. Following this initial report, a number of other studies have confirmed the finding, although the reported incidence of hypertension in hyperparathyroidism has varied between 18 and 78% (5-11). Of even greater significance, however, were reports that, following parathyroidectomy in hyperparathyroid patients, hypertension often improved (5,6,9-11). In spontaneously hypertensive rats (SHR), parathyroidectomy was also found to attenuate hypertension (12-14). This observation, together with reported increased levels of parathyroid hormone (PTH) in SHR (15,16), led to the suggestion that PTH might be a "hypertensive" hormone, and that hypersecretion of the hormone might be responsible for a portion of essential hypertension (13,14,17,18). This suggestion was seemingly supported by a second series of findings.

For many years, an inverse relationship between dietary calcium and blood pressure had been described (19-21). In 1983, the first prospective study was published showing that supplemental dietary calcium could decrease blood pressure in hypertensive patients (22). As PTH secretion is normally inhibited by increased calcium, such a finding could be taken as further evidence for the involvement of PTH in hypertension. Following this initial report, a number of other interventional studies of calcium in hypertension were undertaken. Some studies supported an anti-hypertensive role for calcium (23,24), whereas other studies showed no effect of calcium on blood pressure (25-27). Thus, the role of dietary calcium in hypertension remained unclear until, in 1988, Lyle *et al.* published a report on characteristics of hypertensive patients who did and did not respond to supplemental calcium (28). Those findings showed that patients with elevated PTH levels were more likely to respond favorably to dietary calcium. Again, this was taken to indicate a causative role for PTH in hypertension. In SHR, which are known to have elevated PTH levels, as previously discussed, a high calcium diet was also shown to be effective in lowering blood pressure (15,29,30). On the basis of these studies, attention turned towards the *in vitro* actions of PTH.

In order to initiate vascular smooth muscle contraction, the free intracellular calcium level must increase. The available calcium then binds to troponin, allowing actin and myosin to interact and resulting in the cascade of events leading to muscle contraction. The effect of PTH on intracellular calcium was therefore studied. Results of these studies showed, by a number of techniques, that PTH could increase

intracellular calcium levels in red blood cells (31), hepatocytes (32) and cardiac myocytes (33). Again, apparent support for the role of PTH in hypertension was thereby obtained.

Despite all the evidence implicating the parathyroid gland and PTH as playing a causative role in some forms of hypertension, a number of perplexing questions remained. First, PTH has always been shown to have hypotensive properties (34,35). Moreover, Pang *et al.* have shown that PTH is directly vasorelaxant (36) and that such an action occurs as a result of blocking the L-type calcium channels in vascular smooth muscle (37). This action is similar to that of calcium channel blockers, such as nifedipine, diltiazen and verapamil, all of which are used in the treatment of hypertension. Thus, PTH, rather than increasing intracellular calcium decreased it. In further support against a hypertensive role for PTH, no reports exist of PTH ever having had a direct vasoconstrictive or hypertensive effect in the absence of significant hypercalcemia. In addition, not all human hyperparathyroid patients are hypertensive, as would be expected if PTH were a vasoconstrictor. Similarly, following parathyroidectomy in hypertensive patients, blood pressure does not uniformly fall -- in some cases, blood pressure remains unchanged or even increases (5,6,8-11). Thus, a situation existed wherein the parathyroid gland was thought to be important in the genesis of hypertension, yet its only known hormonal product, PTH, was shown to be hypotensive. Furthermore, a paradox also existed whereby both supplemental dietary calcium and calcium channel blockers were shown to be effective in treating hypertension. The field of calcium and hypertension was thus quite confused by the

mid-1980's.

In 1986, the first data which could, we felt, potentially provide a resolution to the above controversies came to light. In that year, Resnick *et al.* published a paper showing an association between renin profile and calcium regulatory hormones (38). He and his colleagues showed that low-renin hypertensive patients, representing approximately 30 to 40% of all hypertensive patients (39), had high levels of PTH but low levels of ionized calcium. Thus, a link was established between low-renin states and an abnormal calcium regulatory hormone profile. It is of significance that this same low-renin group is the one that responds favorably to dietary calcium (40). More important, however, was the association of a high PTH level and low ionized calcium. This relationship was of significance because, if PTH levels were primarily elevated in hypertension, ionized calcium should be increased, not decreased! The fact that ionized calcium was low in the face of increased PTH was taken as evidence that elevated PTH was secondary to the low calcium. Since this observation, Resnick has explained the low ionized calcium level as being due to a relative increase in intracellular calcium entry in hypertension at the expense of extracellular levels (38). Indeed, increased intracellular calcium levels have been reported in various cell types from hypertensive humans (41-44) and animals (42,45-47). Others have suggested that the low ionized calcium is due to a renal calcium leak, and have shown such a phenomenon in some hypertensive humans (48) and animals (15,49). Nevertheless, if PTH is functioning appropriately, it should be able to maintain a normal calcium level, albeit at the expense of increased PTH levels. But this is not the case -- ionized

calcium remains low in hypertensive patients despite increased PTH levels. In reviewing the literature, it became apparent that the hypercalcemic action of PTH was not the only PTH-related process that appeared to be inhibited. In the presence of elevated PTH, one would expect to see an increase in other PTH-dependent processes, such as urinary calcium reabsorption, urinary cAMP generation, and intestinal calcium absorption. In fact, such processes are actually attenuated in cases of hypertension associated with elevated PTH (15,48,50-52). Thus, immunoreactive levels of PTH are not matched by the expected PTH bioactivity. This suggested to us that either an inactive form of PTH was being measured in hypertension, that the tissues were subnormally responsive to normal PTH, or that an inhibitor of PTH action was present in hypertension. With regard to the former hypothesis, Ganten suggested that N-terminal deleted fragments of PTH might have hypertensive properties (53). In previous studies, however, it was not possible to show any vascular effects of such fragments (54). With respect to the suggestion of altered tissue sensitivity to PTH, this was thought to be less likely, as no reports of an altered PTH/ionized calcium relationship had been described antedating the onset of hypertension. Thus, we considered the possibility of a circulating factor inhibiting the action of PTH and possibly affecting blood pressure.

The concept of circulating vasoactive substances was not new. As early as 1959, Dahl demonstrated that parabiosis of a normotensive and a hypertensive rat resulted in an increase in blood pressure in the normotensive animal (55). Subsequent studies by Zidek *et al.* showed that cross-circulation between normotensive

and hypertensive rats could increase blood pressure in the normotensive animal (56). Wright and McCumbee showed that red blood cells from SHR contained an extractable fraction which could increase blood pressure when injected into normotensive rats (57). Other investigators have shown that serum from hypertensive rats can sensitize normotensive rats or arterial strips to the actions of various pressor agents (58-61). It was, therefore, felt that a circulating hypertensive factor could exist in some hypertensive animals and humans, and that such a factor might inhibit the action of PTH. The following hypothesis was thus created: "A circulating factor exists in the plasma of certain hypertensive humans and animals which increases blood pressure by increasing intracellular calcium levels in vascular smooth muscle. Such a factor might be produced by the parathyroid gland, and might thus be suppressed by dietary calcium". This hypothesis had the potential to explain the involvement of the parathyroid gland, but not PTH, in hypertension. It could explain why dietary calcium was effective in hypertension, and why calcium channel blockers were also effective under the same circumstances. This factor would be associated with reported states of altered calcium balance in hypertension -- namely the low-renin type of hypertension. As low-renin hypertension is also associated with salt sensitivity, the factor should also be related to the salt-sensitive state.

This work then took the following course. The SHR, showing the abnormal calcium regulatory profile of low-renin hypertension, was used as a model. In Chapter I, the presence of a circulating hypertensive factor in SHR plasma is described. The hypertensive action of this factor is further shown to correlate temporally with an

increase in calcium uptake in vascular smooth muscle, suggesting that the factor may act by increasing intracellular calcium levels. In Chapter II, the effect of the factor on potentiating the action of several vasopressors is described. This again suggested that this factor might act by increasing intracellular calcium levels in vascular smooth muscle. In Chapter III, the effect of the factor in inhibiting the vascular actions of PTH is described. In Chapter IV, the stimulatory effect of the factor on Na^+-K^+ ATPase is described in order to show that it is distinct from putative ouabain-like factors in hypertension. In Chapter V, calcium channel blockers are shown to block the hypertensive effect of the factor, again suggesting that the factor acts by increasing intracellular calcium in vascular smooth muscle. Chapter VI shows that a low calcium diet stimulates, and a high calcium diet inhibits, expression of the factor. Chapter VII presents the results of a cross-transplantation experiment showing that hypertension and the hypertensive factor track with SHR parathyroid glands, rather than with the animal. In addition, parathyroidectomy decreased blood pressure in SHR rats and resulted in disappearance of the hypertensive factor activity from the plasma. On this basis, we named the factor "Parathyroid Hypertensive Factor", or "PHF" for short. In Chapter VIII, PHF-like activity is reported in human essential hypertension, and in Chapter IX, PHF is shown to be associated with some hypertensive hyperparathyroid patients, but not with normotensive hyperparathyroid patients. Post-parathyroidectomy, PHF-like activity disappears from the plasma, suggesting that the parathyroid gland may also be a source of PHF in humans. Chapters X and XI confirm the association of PHF with low-renin and salt-sensitive

hypertensive models. These chapters comprise the thesis, but do not represent the entire work. A number of studies have either been carried out in collaboration or are underway showing that PHF is present in low-renin, salt-sensitive patients (62), that PHF inhibits urinary sodium excretion, possibly offering the basis for an explanation of the phenomenon of salt-sensitivity (63), and that PHF does, in fact, result in a measurable increase in free intracellular calcium (64). PHF has been purified largely through the efforts of Dr. Christina Benishin (65), the parathyroid origin confirmed in SHR by gland culture techniques (66), and an antibody to PHF raised which shows no cross-reactivity to any form of PTH (67) or substance produced from the parathyroid gland, such as chromogranin, PTHrp or ubiquitin. Thus, PHF does appear to be a definite entity, offering an explanation for a number of controversies in the field of hypertension. Certainly, years of work lie ahead to fully understand the role of PHF in hypertension.

The chapters that follow, for the most part, represent papers either published or submitted for publication. Much of the discussion in these chapters is therefore somewhat repetitive. For this reason, the reader may find it advantageous to primarily refer to the thesis Introduction and Discussion in order to put the work into context.

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I. EFFECTS OF SPONTANEOUSLY HYPERTENSIVE RAT PLASMA ON BLOOD PRESSURE AND TAIL ARTERY CALCIUM UPTAKE IN NORMOTENSIVE RATS

A. INTRODUCTION

The etiology of essential hypertension remains unknown. A number of studies have suggested that one or more circulating factors may play a role in the genesis or maintenance of hypertension (1-6). In genetically hypertensive rats, evidence for such a circulating factor has been obtained by a number of methods. In parabiosis (2,3) and cross-circulation (5) experiments, an increase in blood pressure could be induced in normotensive animals by exposure to blood from hypertensive animals. Subcutaneous injection of a spontaneously hypertensive rat (SHR) erythrocyte-associated factor has also been shown to induce hypertension in normotensive Wistar-Kyoto (WKY) rats (1). Similarly, an increase in blood pressure could be induced in normotensive Dahl salt-sensitive rats by chronic injection of serum from hypertensive Dahl salt-sensitive rats (6).

In addition to the preceding studies, there are reports of circulating factors in

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both hypertensive rats and hypertensive humans that increase intracellular calcium (7-11). Such findings have been reported for platelets (7,9,10) and neutrophils (8) incubated in the presence of serum from humans with essential hypertension, as well as for platelets (10) and aortas (11) incubated in the presence of SHR serum.

As vascular tone is influenced by the level of intracellular calcium, it would seem reasonable to assume that factors that increase blood pressure and factors that increase intracellular calcium may be related. Upon review of the literature, however, it became apparent that there was a relative lack of evidence correlating circulating plasma factors that increase the calcium uptake of vascular tissue with those factors that increase blood pressure. The only report of such a relationship was for a hypertensive, erythrocyte-associated substance that increased the calcium uptake of rat aortic rings (1). In addition, it seemed unusual that long-term exposure to hypertensive serum was usually required in order to increase the blood pressure in normotensive animals, yet only minutes were required for hypertensive serum to raise intracellular calcium. One might expect that a substance that rapidly increased intracellular calcium would also rapidly increase blood pressure.

With these questions in mind, it was our purpose to search for evidence of a substance that could increase intracellular calcium in vascular smooth muscle and that could also increase blood pressure when acutely administered to normotensive rats.

B. METHODS

Male SHR, WKY and Sprague-Dawley (SD) rats weighing between 300 g and 350 g were used. Plasma from these rats was prepared in the following manner. Blood was obtained by decapitation, heparinized (10 IU/ml) and centrifuged for 10 minutes at 3,000 g. The resultant plasma was removed and dialyzed (molecular weight cut-off 1,000) against distilled water for 24 hours at 4°C. Plasma was then removed and stored at -20°C until used. For one series of experiments, the plasma was briefly boiled in order to determine the effects on the pressor activity of the plasma.

Effect of SHR Plasma on Normotensive Rats

Rats were anesthetized with sodium pentobarbital (50 mg/kg) intraperitoneally. Plasma was then administered via the jugular vein either as a bolus of 2 mL/kg or as a continuous infusion of 1.2 mL/kg/hour. Blood pressure was continuously recorded via a catheter in a carotid artery. Plasma infusion was carried out for 90 minutes -- a time that resulted in a maximal pressor response. After 90 minutes, normotensive plasma was substituted in the infusion. In another set of experiments, varying bolus doses of SHR plasma were administered and the blood pressure responses were recorded.

^{45}Ca Uptake

The effect of SHR and WKY plasma on the *in vitro* uptake of ^{45}Ca by vascular

tissue was studied in the rat tail artery using the low-affinity lanthanum-resistant Ca pool assay for the determination of intracellular calcium uptake, as previously described (12). Briefly, longitudinal rat tail artery strips were equilibrated for 2 hours at 37°C in oxygenated tubes containing modified Krebs-Henseleit (K-H) buffer. After this equilibration period, the tissue was incubated for 6 minutes in the presence of test plasma (30% by volume in K-H buffer) and ^{45}Ca. At the end of this incubation, the strips were rinsed in ice-cold Ca-free K-H buffer and then incubated in ice-cold La solution for 15 minutes. The strips were then blotted, weighed and digested overnight in a mixture of 30 μl glacial acetic acid and 100 μl methylbenzethonium hydroxide. The samples were then counted in a scintillation counter, and the calcium uptake was determined.

In separate experiments, the effects of varying concentrations of SHR plasma and the effects of varying incubation times in the presence of 30% SHR plasma were examined. Concentrations used in the former experiments were 10%, 30% and 50% plasma in K-H buffer, and incubation times in the latter experiments were 6, 30, 45 and 60 minutes.

Statistics

Significance of responses within groups of individual rats was assessed using Student's *t* test for paired data. Comparisons among experimental groups were carried out using analysis of variance, and comparisons between any two treatments were carried out using Student's *t* test for unpaired data. A $P > .05$ was considered

not significant.

C. RESULTS

Plasma Infusion

Baseline mean arterial pressures were similar in SD and WKY rats (118±2 mm Hg versus 123±3 mm Hg, mean ±SEM, P=.29). The time courses for mean changes in blood pressure after infusion and bolus administration of test plasma into SD rats are given in Figures I.1 and I.2. In both cases (infusion and bolus), SHR plasma caused a significant increase in blood pressure. SD and WKY plasma, on the other hand, had no significant effects on blood pressure.

In the case of SHR plasma infusion, blood pressure was significantly increased by 45 minutes, with a peak effect being reached by 90 minutes. Infusion of plasma past this time did not result in a further increase in blood pressure. Approximately 10 minutes after the infusion was changed to normotensive plasma, blood pressure began to drop, reaching preinfusion levels in approximately 30 minutes.

Bolus injection of SHR plasma resulted in a more rapid increase in blood pressure, with an initial significant increase being noted at 30 minutes and the peak occurring at 45 minutes. By 90 minutes, the blood pressure had returned to baseline.

Time courses were identical when WKY rats were used as the experimental animal (results not shown). The magnitude of the blood pressure increase caused by SHR plasma was similar in both WKY and SD rats for both infusion (P=.65) and bolus administration (P=.91) (Figures I.3 and I.4).

Figure I.5 shows the dose-dependent hypertensive effect of SHR plasma. Although the mean increase in blood pressure rises steadily as the dose increases, a significant increase in blood pressure is not noted until 1.0 ml of plasma is given.

The time courses for changes in blood pressure using crude, dialyzed, and boiled and dialyzed SHR plasma are given in Figure I.6. The hypertensive response to each of these differently treated plasma extracts was equivalent.

^{45}Ca Uptake

^{45}Ca uptake for SD tail arteries incubated in the presence of buffer, WKY and SHR plasma is given in Figure I.7. These results show that SHR plasma significantly increased ^{45}Ca uptake compared to control, but that WKY plasma had no effect. Figure I.8 shows the dose-dependent effect of SHR plasma on ^{45}Ca uptake. Increased doses of SHR plasma result in increasing ^{45}Ca uptake. Figure I.9 shows the effect of incubation time in the presence of SHR plasma on ^{45}Ca uptake in WKY tail arteries. From this figure, it is evident that maximal ^{45}Ca uptake occurs at approximately 45 minutes of incubation time -- the same time at which the maximal pressure response was noted to occur in bolus administration experiments.

D. DISCUSSION

This study demonstrates that SHR plasma possesses the ability to increase blood pressure in normotensive rats when administered intravenously. The pressor response is delayed but corresponds to the observed time-dependent increase in

calcium uptake displayed by the vascular tissue. Presumably the increase in intracellular calcium is responsible for the hypertensive effect. The dose-dependent effects for both the hypertensive and calcemic properties of SHR plasma suggest that a specific factor in the plasma may be causing the observed effects. This factor would be heat stable and have a molecular weight of greater than 1,000.

Although many reports of circulating hypertensive factors in hypertensive rats exist (1-6), evidence for such substances has often been indirect. Levels of substances, such as ouabain-like factors, have been correlated with the degree of hypertension (13-15), but evidence that such substances can directly cause a hypertensive response when given intravenously is relatively lacking. Alternatively, serum from SHR rats has been shown to induce an increased sensitivity to pressure agents in normotensive rats (16-18) but not to increase blood pressure directly. In those studies that have demonstrated an increase in blood pressure, it has usually required repeated injections of hypertensive serum, and a number of days, for hypertension to develop (1,6). An exception was a cross-circulation experiment performed by Zidek *et al.* (5), where hypertension developed in the normotensive rats by 1 hour. This latter time course is, in fact, similar to that reported in this study.

One might wonder why findings such as ours have not been widely reported. Certainly one would expect that similar experiments with direct administration of hypertensive plasma would have been carried out in the past. Although thoughts as to why such results have not been previously obtained are purely speculative, one possible explanation might be that previous investigators allowed inadequate time for

a pressor response to occur. Although blood pressure began to increase about 15 minutes after bolus administration of SHR plasma, a statistically significant increase was not noted until 30 minutes. Similarly, with infusion experiments, a statistically significant result was not noted until 45 minutes. In the latter case, however, the development of a pressure response is presumably somewhat dependent on the infusion rate. In any case, the time lag certainly raises questions as to the nature of the hypertensive factor.

Conventional endogenous pressor agents, such as norepinephrine, angiotensin II and vasopressin, are well known to induce an immediate and rapid rise in blood pressure. We, in fact, confirmed this by giving these substances, as well as ouabain, to normotensive rats. In all cases, blood pressure increased immediately after injection, peaked rapidly, and returned to baseline rapidly. The observed time lag in pressor response with the hypertensive plasma would thus tend to rule out conventional pressors as a cause of the hypertensive effect. Furthermore, the plasma used in this study was dialyzed at a molecular weight cut-off of 1,000. This procedure should have removed vasoactive ions such as calcium and most of the substances mentioned above, including the ouabain-like factors that have a reported molecular weight in the range of 400 to 500 (19,20). One possible explanation for the time lag, especially in infusion experiments, could be that a volume effect was being seen. This does not seem to be the case, as blood pressure returned to baseline shortly after the substitution of normotensive plasma, in place of SHR plasma, in the infusion. Moreover, one would have expected an immediate increase in blood pressure in bolus

administration experiments if a volume effect was being observed. Finally, plasma from normotensive rats given in similar volumes had no effect on blood pressure in either infusion or bolus administration experiments. Another consideration is that hypertension may have developed in the normotensive rats as a non-specific response to the presence of plasma from a different rat strain. Again, this does not seem to be the case, as WKY plasma administered to SD rats had no effect on blood pressure. Similarly, a dose-response effect was noted in the normotensive rats for both the hypertensive effect of SHR plasma and for the increase in ^{45}Ca uptake. Such an effect would not be expected if a non-specific effect was being seen. What is more likely is that a secondary process is necessary for the development of hypertension. Such a process could involve protein synthesis or membrane alterations. Currently, we are investigating the effect of the hypertensive plasma on cellular and membrane functions in order to try and elucidate the mechanism of action.

Previous studies on circulating hypertensive factors have tended to be either entirely *in vivo* or entirely *in vitro*. This study has involved both components. Although we have no way of knowing whether one or more circulating factors is responsible for both the hypertensive and the cellular calciotropic effects of SHR plasma, it would seem reasonable to expect that a substance that increases intracellular calcium in vascular smooth muscle would also cause an increase in blood pressure. This presumption is supported by the identical time courses observed for both the calciotropic and hypertensive effects of the plasma. We therefore hypothesize that a single factor is responsible for both effects. Presumably, this factor

increases intracellular calcium in vascular smooth muscle, causing an increase in vascular tone, resulting in an increase in blood pressure. A similar relationship has likewise been described by Wright and McCumbee (1), who demonstrated increased ^{45}Ca uptake in rat aortic rings incubated in the presence of an erythrocyte membrane-associated factor that had also been shown to have pressor effects. This erythrocyte-associated factor could be easily dissociated from the membrane and, in its dissociated form, induced significant hypertension in rats within 1 day of subcutaneous administration. It is not stated whether this substance could also be found in the plasma in their study, but the possibility exists that the substance described by them and the substance in this study could be similar. Further work will be needed, however, to characterize this and other hypertensive factors.

The results of this study may also help to explain the reported increase in responsiveness of normotensive rats to norepinephrine after administration of hypertensive serum or plasma (16,18). As the action of norepinephrine is dependent upon intracellular calcium levels, such observations could be explained by an increase in intracellular calcium caused by a factor in the plasma, as observed in this study. Interestingly, Battarbee *et al.* (18) reported that maximum potentiation of the pressor response to norepinephrine in normotensive rats occurred 30 minutes after the administration of hypertensive serum. This delay is similar to the delay we observed in the pressor response after bolus administration of SHR plasma to normotensive rats. These findings may suggest that the same, or a similar, factor is being identified in both studies.

In conclusion, this study confirms the presence of a circulating hypertensive factor in SHR rats. It uniquely shows that direct intravenous administration of SHR plasma can cause a semi-acute, reversible increase in blood pressure in a dose-dependent manner. This study also uniquely demonstrates that a circulating factor from SHR rats can increase intracellular calcium in the arteries of normotensive rats, again in a dose-dependent manner and with a time course corresponding to the pressor response. Such findings may explain the increased sensitivity of SHR rats to cellular calcium-dependent vasopressors (21,22), as well as earlier reports of circulating substances in hypertensive rats that sensitize normotensive rats to the same vasopressors.

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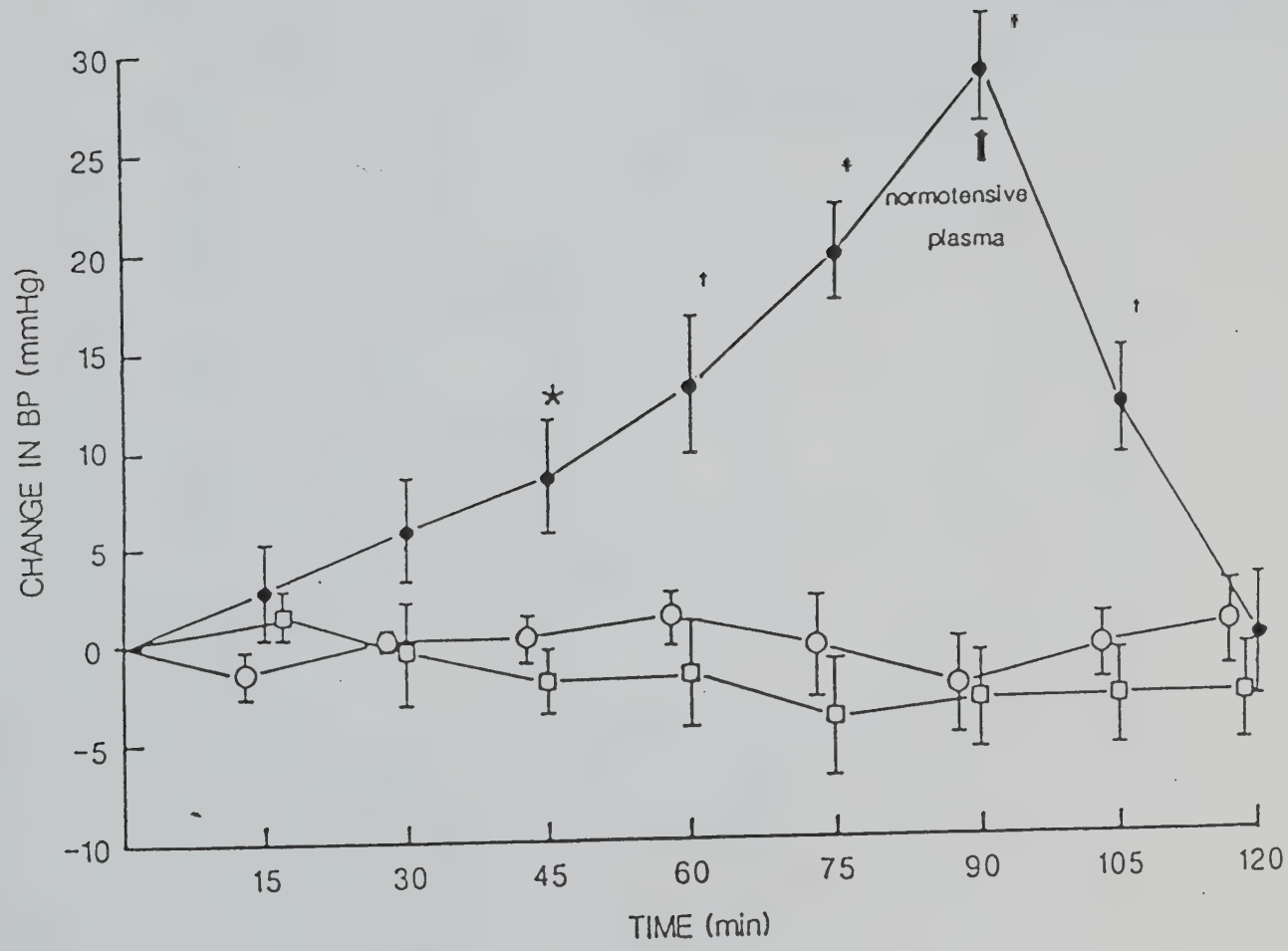

Figure I.1.

Change in mean arterial pressure during infusion of SHR (•, n=12), WKY (o , n=8) and SD (□, n=6) plasma into SD rats. Arrow indicates substitution of SD plasma for SHR plasma. Means±SEM are given. *$P<.05$ for difference from baseline pressure. †$P<.01$ for difference from baseline pressure. ‡$P<.001$ for difference from baseline pressure.

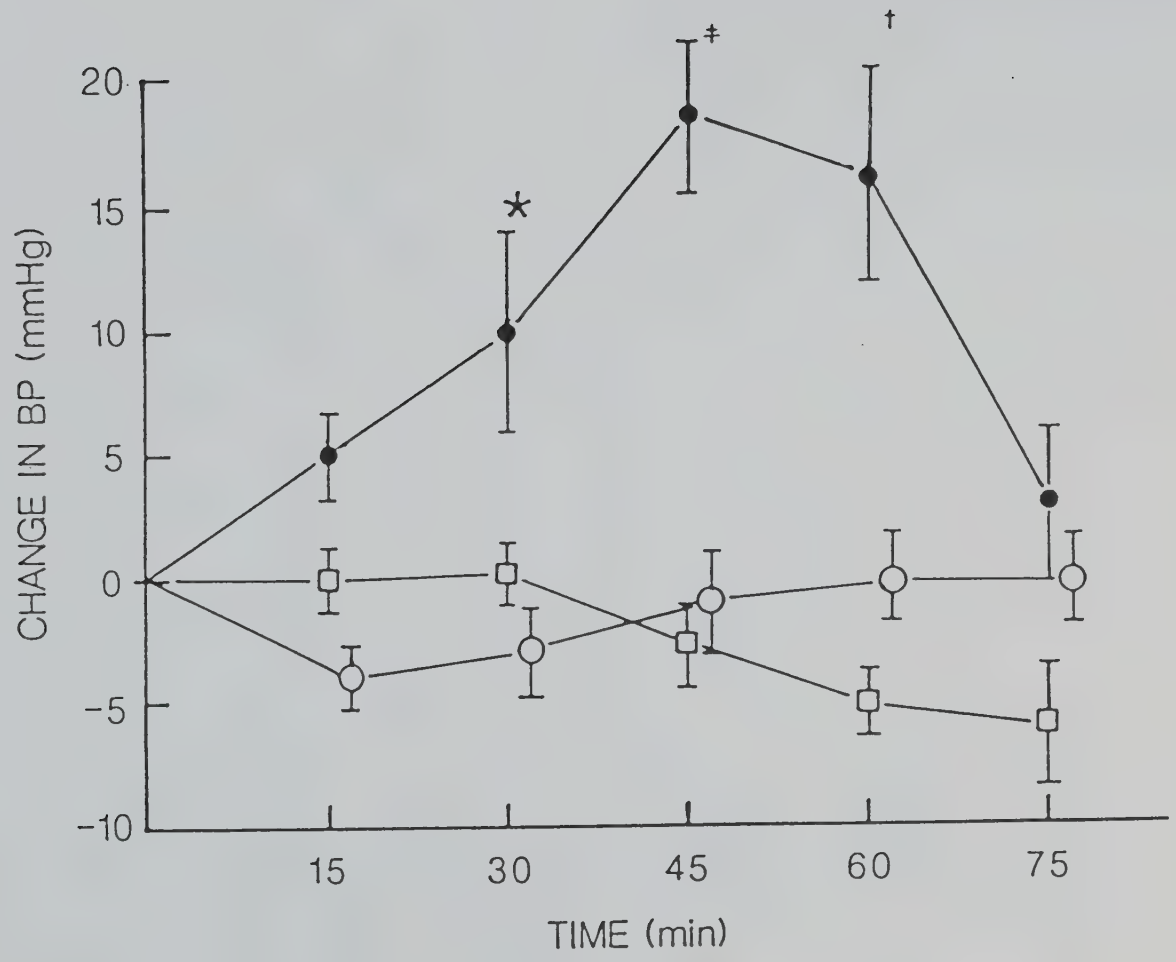

Figure I.2.

Change in mean arterial pressure after bolus administration of SHR (•, n=9) and SD (□, n=6) plasma into SD rats. Means±SEM are given. *$P<.05$ for difference from baseline pressure. †$P<.01$ for difference from baseline pressure. ‡$P<.001$ for difference from baseline pressure.

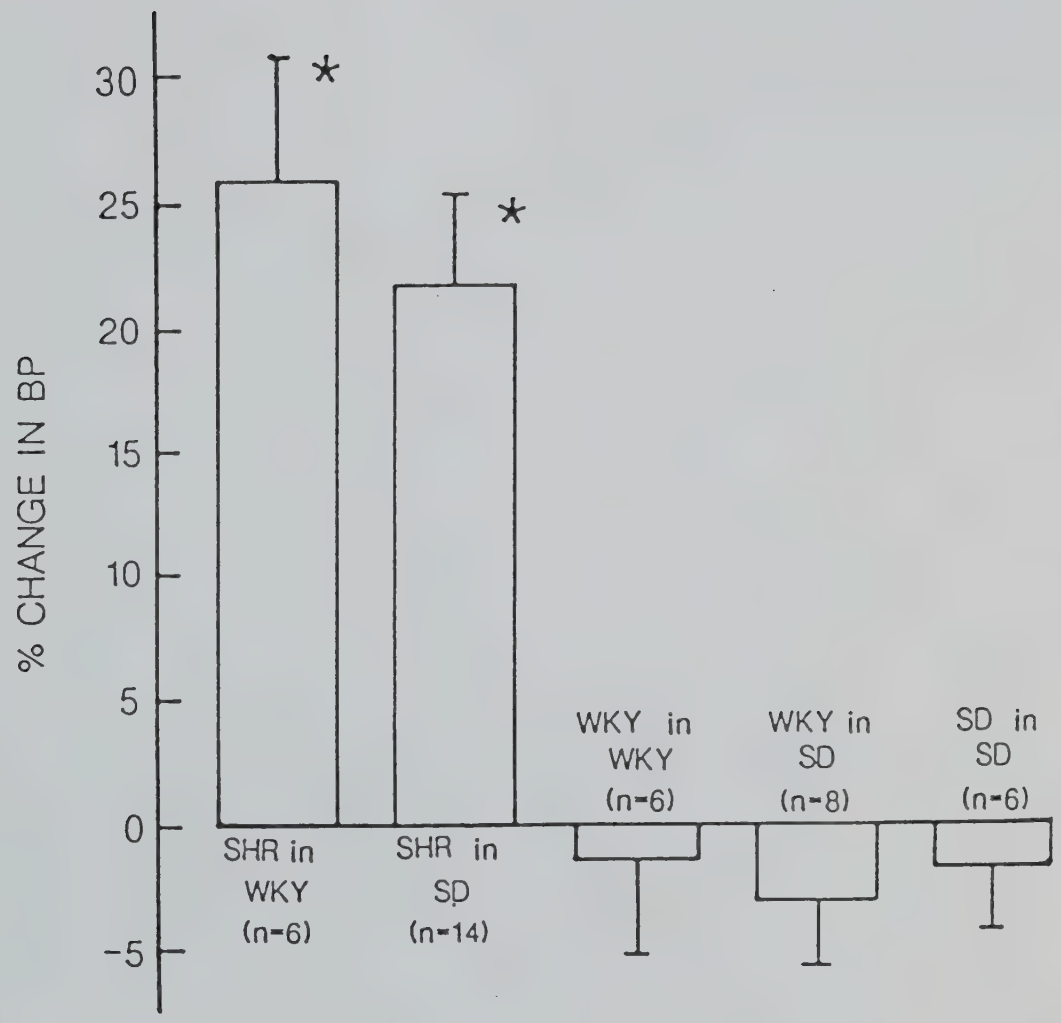

Figure I.3.

Maximal change in blood pressure with infusion of indicated plasma. Means $\pm$ SD are given. $^*P < .001$ for difference from baseline pressure.

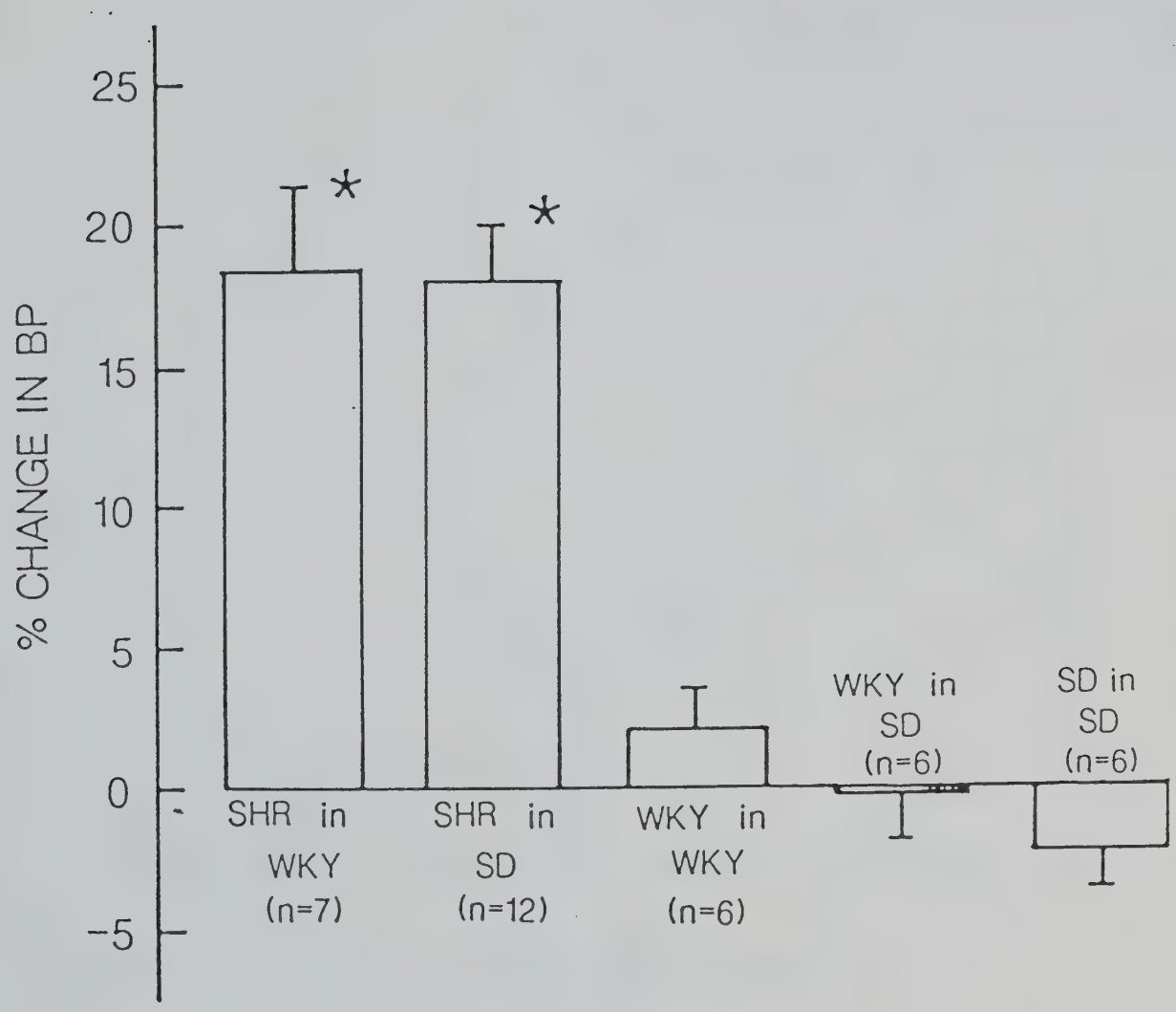

Figure I.4.

Maximal change in blood pressure with bolus administration of plasma into indicated rats. Means$\pm$SD are given. *$P < .001$ for difference from baseline pressure.

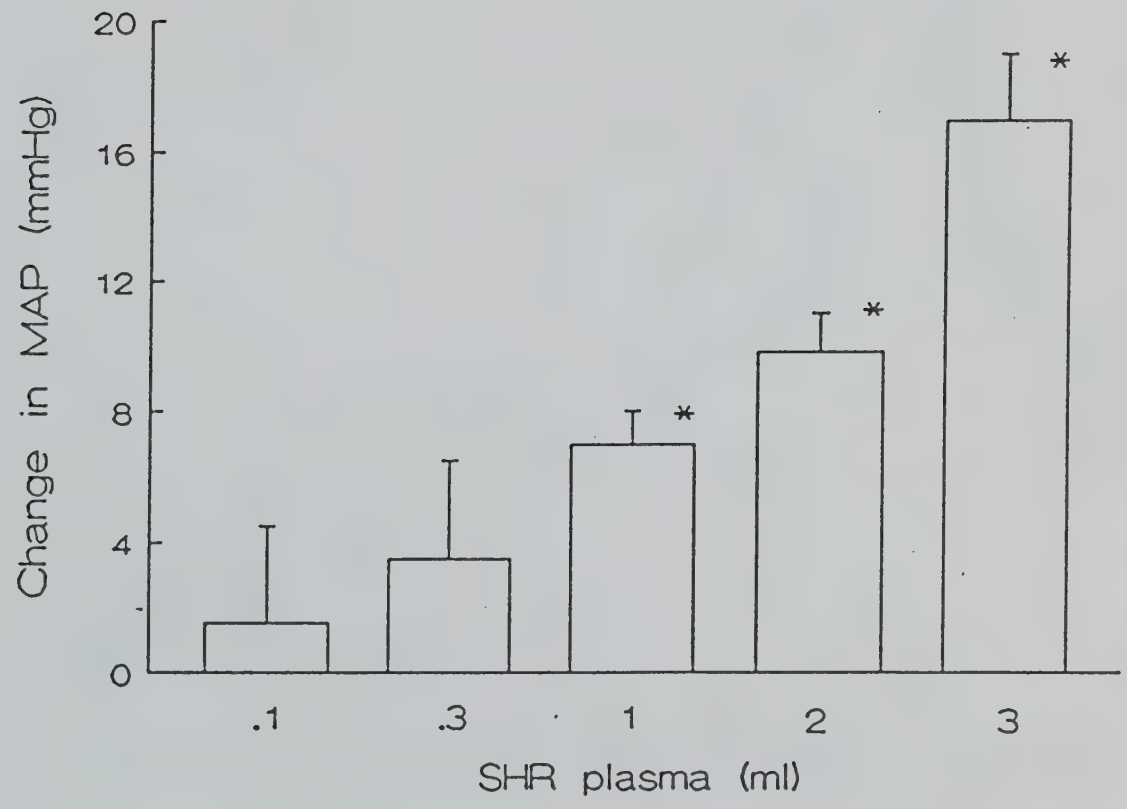

Figure I.5.

Maximal blood pressure response to varying bolus doses of SHR plasma (n=7). Means±SEM are given. *$P < .001$ for difference from baseline pressure.

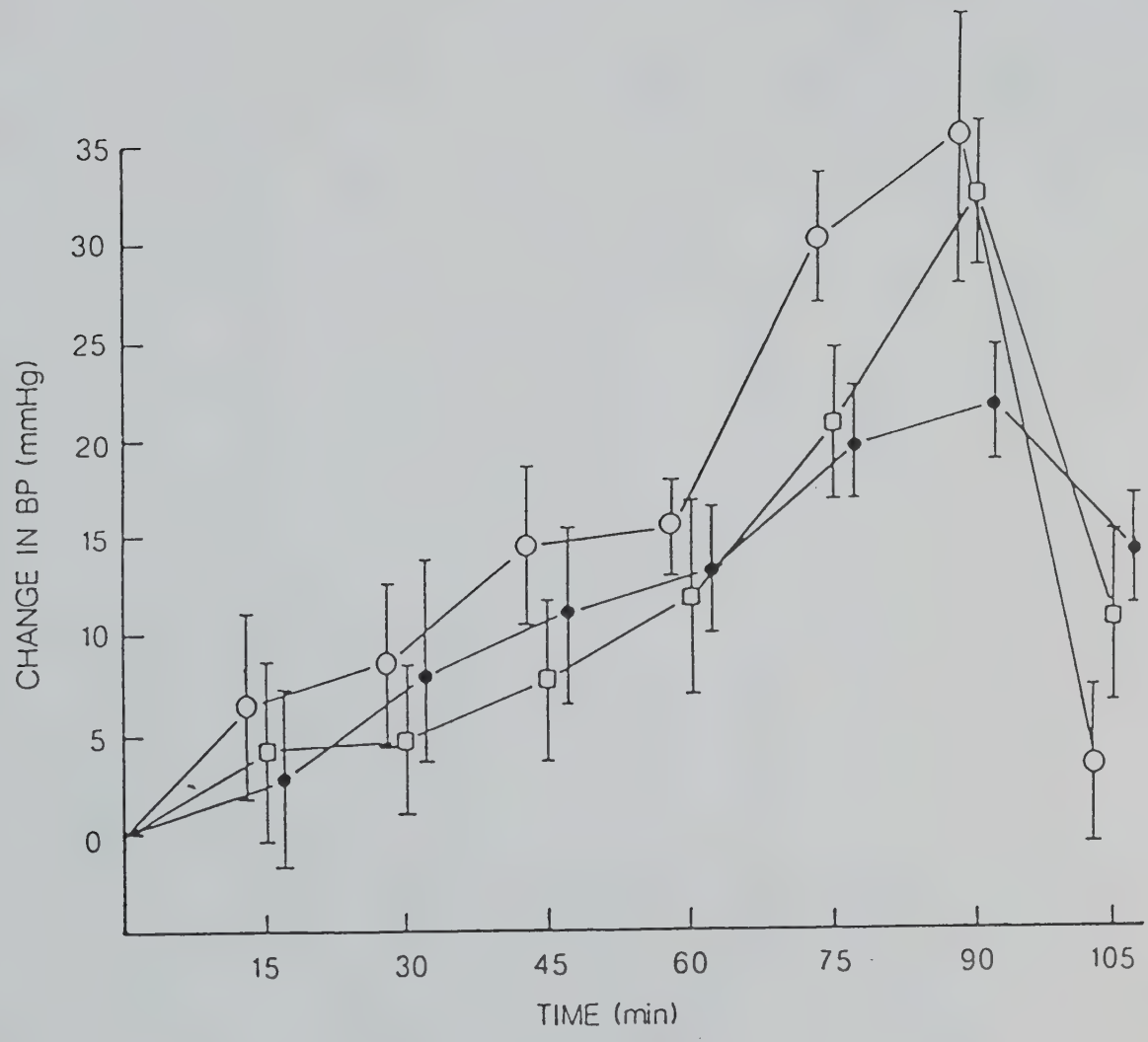

Figure I.6.

Effect of crude (□, n-7), dialyzed (o , n=5) and boiled and dialyzed (•, n=4) SHR plasma infusion on blood pressure in the SD rat. Means±SEM are given. At 90 minutes, control plasma was substituted in place of hypertensive plasma. Difference between values at any given time are not significantly different ($P<.05$).

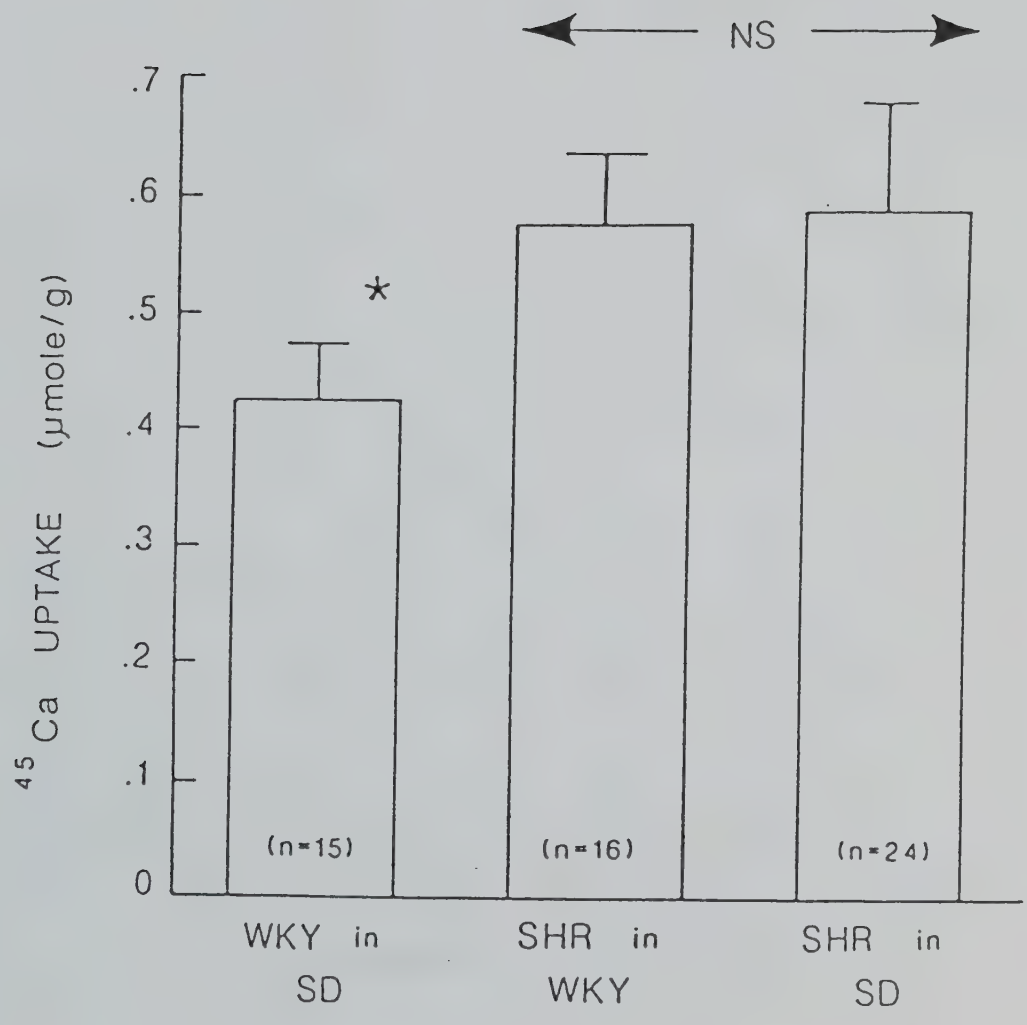

Figure I.7.

^{45}Ca uptake in response to indicated plasma in indicated rat tail artery preparation, respectively. Means±SD are given. NS=no significant change. *$P<.001$ for difference in ^{45}Ca uptake between WKY and SHR plasma.

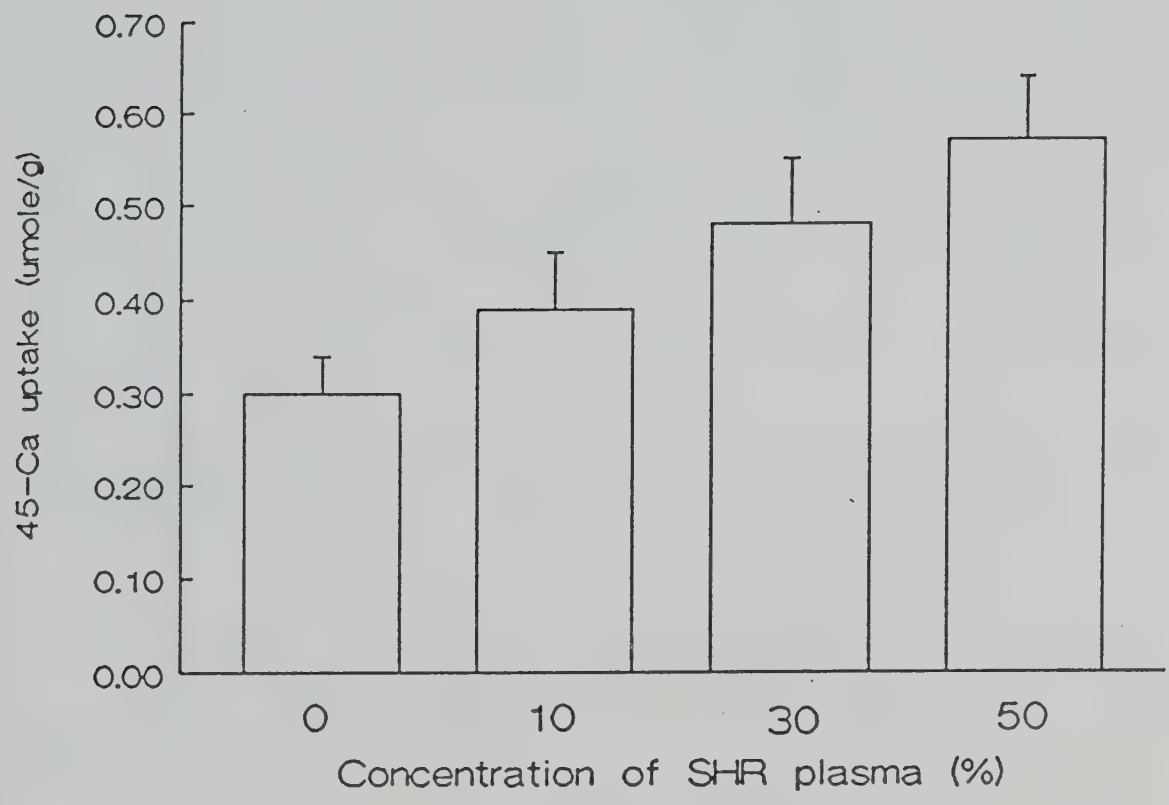

Figure I.8.

^{45}Ca uptake in SD tail arteries in response to varying concentrations of SHR plasma (n=16). Means±SD are given. All values are significantly different from control (0%) ($P<.001$).

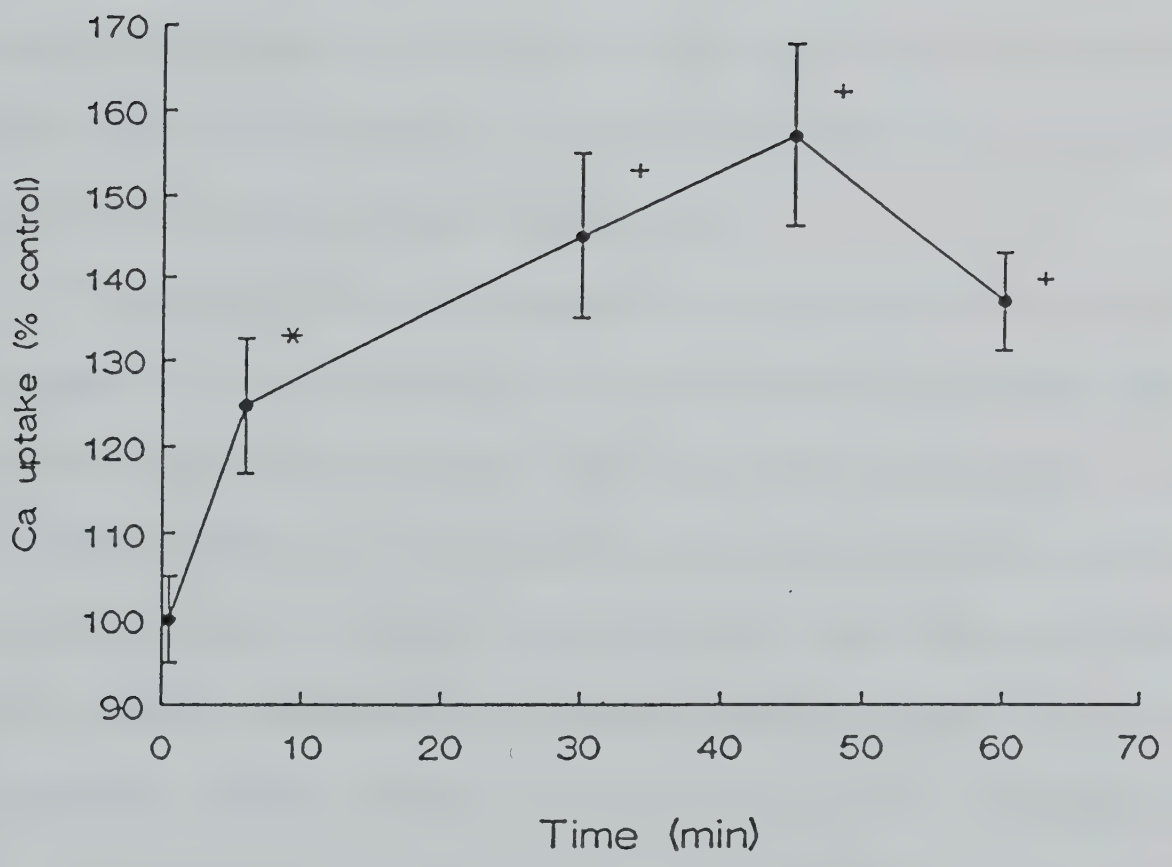

Figure I.9.

Effect of incubation time in the presence of SHR plasma on ^{45}Ca uptake in WKY tail arteries (n = 12). Means ± SEM are given. *$P < .05$ for difference from control. †$P < .01$ for difference from control.

II. *IN VIVO* POTENTIATION OF VASOPRESSORS BY SPONTANEOUSLY HYPERTENSIVE RAT PLASMA: CORRELATION WITH BLOOD PRESSURE AND CALCIUM UPTAKE

A. INTRODUCTION

Various abnormalities have been described in essential hypertension. One of these abnormalities -- an increased sensitivity to endogenous pressors -- has been reported in both human hypertensives (1-3) and animal models (4-7) of hypertension. Further studies have suggested that this increased sensitivity to pressor agents may be transmissible through serum or plasma (8-11).

The possible existence of humorally-transmitted hypertensive factors has been addressed by a number of studies. These studies have shown that hypertension can be induced in normotensive animals receiving injections of hypertensive serum (12-14), red blood cell extracts (15) or linked in cross-circulation (16). Other studies have shown that intracellular calcium can be increased *in vitro* in platelets (17,18), and white blood cells (19) from normotensive humans by incubation in plasma from a hypertensives. All these preceding studies strongly suggest that circulating hyper

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tensive factor may be important in essential hypertension. Recently, we have described the presence of a novel hypertensive factor in the plasma of spontaneously hypertensive rats (SHR) (20). This factor increases blood pressure in a delayed, dose-dependent manner when given intravenously to normotensive rats. It also increases calcium uptake in vascular smooth muscle of normotensive rats *in vitro*. As many endogenous pressors act largely by increasing the free intracellular calcium concentrations in vascular smooth muscle, we speculated that this hypertensive substance might also induce an increase in sensitivity to calcium-dependent pressors *in vivo*. The purpose of this study, therefore, was to examine the pressor response to noradrenaline, arginine vasopressin and angiotensin II in normotensive rats in the presence of SHR plasma.

B. METHODS

Rats and Rat Plasma

Male SHR, Sprague-Dawley (SD) and Wistar-Kyoto (WKY) rats weighing 300 to 350 g were used for this study. To prepare plasma, blood was obtained by decapitation, heparinized (10 IU/ml) and centrifuged at 3,000×G at 4°C for 10 minutes. The resultant plasma was then dialyzed against saline for 24 hours at a molecular weight cut-off of 1,000 daltons. Plasma was used immediately or stored at -20°C for a maximum of 2 days before use.

Isolation and Potentiation Studies

SD rats were anesthetized (pentobarbital, 50 mg/kg intraperitoneally) and a jugular vein and carotid artery cannulated. Mean arterial pressure (MAP) was continuously monitored via the catheter in the carotid artery during the experiments. For blood pressure studies, 2.5 ml/kg of SHR or WKY plasma was given as a bolus intravenous injection via the jugular vein. This dose of plasma was chosen based on previous dose-response studies which indicated that an adequate hypertensive effect could be obtained with this dose of plasma, while minimizing the confounding variable of volume expansion. MAP was monitored for 90 minutes following the injection.

For potentiation studies, the following pressors and doses were used: norepinephrine (NE), 0.3 μg/kg, angiotensin II (AII), 120 ng/kg, and arginine vasopressin (AVP), 0.3 μg/kg. All pressors were freshly made up in saline at a concentration resulting in an injected volume of approximately 0.1 ml. In these studies, SD rats were anesthetized and cannulated as described above. After ensuring that a normal and stable blood pressure was present, the rats were given a baseline intravenous dose of the selected pressor agent. After blood pressure returned to baseline, SHR or WKY plasma was given as a bolus intravenous dose of 2.5 mg/kg. Following this, the same dose of the respective pressor was given at 15 minute intervals for NE and AII, and at 20 minutes intervals for AVP. These times were selected as they allowed for adequate recovery of blood pressure between doses and did not result in a progressive decreased responsiveness to the pressor agent in control circumstances.

^{45}Ca Uptake

^{45}Ca uptake was measured in SD tail arteries *in vitro* using the low-affinity lanthanum-resistant pool method as described previously (21). Briefly, tail artery strips were equilibrated in oxygenated (95% O_2, 5% CO_2)Krebs-Henseleit buffer (NaCl, 115 mmol; KCl, 5 mmol; NaH_2PO_4, 1.2 mmol; $MgSO_4$, 1.2 mmol; $CaCl_2$, 2.1 mmol; $NaHCO_3$, 25 mmol; glucose, 11 mmol) for 2 hours. They were then transferred to tubes containing 30% SHR or WKY plasma and 0.1 μCi ^{45}Ca in Krebs buffer, and incubated for varying lengths of time. At the end of the incubation period, the strips were rinsed in ice-cold Ca^{++}-free La^{+++}-containing solution, blotted, weighed and digested overnight in a mixture of benzalkonium chloride (100 μl) and glacial acetic acid (30 μl). The digested tissue samples were then counted in a scintillation counter and the Ca uptake calculated.

Statistics

Differences within individual rats were assessed using Student's *t* test for paired data. Differences between groups of experimental animals were assessed using Student's *t* test for unpaired data or analysis of variance, as appropriate. All means are given as the value$\pm$standard error of the mean (SEM).

C. RESULTS

The initial baseline blood pressure of the SD assay rats was 117$\pm$4 mm Hg. Figure II.1 shows the blood pressure response of SD rats to bolus injection of SHR

or WKY plasma. In this figure, MAP can be seen to increase slowly and peak at 40 to 50 minutes in response to SHR plasma. WKY plasma, on the other hand, had no significant effect on MAP in SD rats.

Figures II.2, II.3 and II.4 show the time course of the pressor responses to NE, AII and AVP, respectively. Mean baseline pressor response to NE was 17±1 mm Hg, to AII 48±3 mm Hg, and to AVP 44±4 mm Hg. A significantly increased pressor effect is noted in the presence of SHR plasma, with peak pressor potentiation occurring at 45 minutes for NE and AII, and at 60 minutes for AVP. Interestingly, the effect of AVP was inhibited at 20 minutes in the presence of SHR plasma. Rats receiving WKY plasma showed no significant pressor potentiation or inhibition.

Figure II.5 shows the time course for ^{45}Ca uptake. In the absence of plasma, ^{45}Ca uptake was maximal by 6 minutes and did not change significantly thereafter. Control (no plasma) ^{45}Ca uptake at 6 minutes was 0.061 μmole/g/wet tissue weight/ minute. ^{45}Ca uptake in the presence of WKY plasma was not different from control values at any time period. In the presence of SHR plasma, ^{45}Ca uptake can be seen to be significantly increased over control values at 6 minutes, with a progression in uptake to 45 minutes.

D. DISCUSSION

In a previous study, we reported the existence of a factor in SHR plasma which increases blood pressure and which increases calcium uptake in vascular smooth muscle (20). This factor is heat stable and has little effect on cardiac output.

In the current study, we confirm the ability of hypertensive plasma to potentiate the actions of various vasopressors, as previously reported by others (8-11). This study, however, is unique in that it correlates a number of previously independent observations. These observations include the ability of hypertensive plasma or serum to increase blood pressure in normotensive animals (12-14,16), the ability of hypertensive plasma or serum to increase the free calcium concentration in platelets or white blood cells (17-19), and the ability of hypertensive plasma to potentiate the action of vasopressors in normotensive animals (8-11). None of these studies examined all the parameters measured, and thus correlation of the various findings was not possible. In the present study, correlation is suggested by almost identical time courses for all three parameters, as noted above. Specifically, peak blood pressure, peak pressor potentiation and peak calcium uptake all occur approximately 45 minutes after plasma injection. This time course is similar to that observed by Zidek *et al.* (16) for a pressor response in their cross-circulation studies. It is also similar to that observed by Battarbee *et al.* (11) for pressor potentiation. To our knowledge, no time course studies have been done on the effects of hypertensive plasma on calcium uptake or on free intracellular calcium concentration. Thus, to summarize, the effects of hypertensive plasma on blood pressure, calcium uptake and pressor potentiation may all be due to the presence of a single factor, as suggested by the similar time course for all three events. Furthermore, we believe that the effect of plasma on pressor potentiation is specific, rather than a non-specific phenomenon related to an increase in blood pressure. This is because similarly acting pressor

agents attenuate, rather than potentiate, each other's actions. Secondly, in models of hypertension, such as aortic coarctation (22) or transplanted pheochromocytoma (23), pressor sensitivity is decreased.

With respect to the early inhibition of AVP action by SHR plasma, we have no ready explanation. Presuming that this effect is not artifactual, the respective properties of AVP and SHR plasma must be such that their interaction results in an early inhibition of the AVP effect. This could conceivably occur at a receptor or post-receptor level by direct or indirect effects.

Although previous studies have shown that hypertensive plasma can increase free intracellular calcium concentrations in platelets (17,18) and white blood cells (19), the current study suggests that this phenomenon may also occur in vascular smooth muscle. In fact, it has been shown that intracellular calcium concentrations are, indeed, increased in the arteries of hypertensive animals (24,25), leading one to speculate whether this phenomenon might be due to the circulating factor described herein. Certainly, given the critical role of calcium in the maintenance of vascular smooth muscle tone and contractility, any factor which increased intracellular calcium levels in vascular smooth muscle might be expected to increase blood pressure as well. In addition, as the pressors used in this study all ultimately act on smooth muscle by increasing the free intracellular calcium concentrations, any substance which increases calcium uptake might also be expected to potentiate the action of these vasopressors. Thus, there is a theoretical basis to suspect that the factor described in this study might act by increasing the calcium concentrations in vascular

smooth muscle.

The actual time course for the observed effects of the plasma is, perhaps, unusual. The fact that the peak effects are noted at about 40 to 60 minutes after plasma injection suggests that the usually considered endogenous pressors, such as catecholamines, angiotensin and vasopressin, are not responsible for the observed effects. Their possible role is further diminished by the fact that the plasma was dialyzed at a 1,000 dalton molecular weight cut-off. This process should have removed most, if not all, of these substances. In addition, ouabain-like factors should have been removed by dialysis, as their molecular weights are reported to be in the 400 to 500 dalton range (26,27). In fact, the dialyzed plasma displayed no ouabain-like effect, as assessed by measurement of ouabain-sensitive ^{86}Rb uptake (unpublished observations).

Of interest is the early significant increase in ^{45}Ca uptake prior to a significant increase in blood pressure or potentiation. This may be due to the fact that blood pressure measurements are much more subject to variability than *in vitro* ^{45}Ca uptake measurements, and thus early minimal increases in MAP may not be apparent. Alternatively, compensatory mechanisms may maintain a stable blood pressure in early stages until the increase in calcium uptake overwhelms such mechanisms. Finally, it is important to note that the low-affinity lanthanum-resistant pool method supposedly only measures calcium influx and does not measure free intracellular calcium levels (28). Although an increased influx of calcium usually results in increased free intracellular levels, it is possible that at an early stage, calcium may be sequestered

in intracellular pools. Similarly, a rapid bidirectional calcium flux could also be taking place. This bidirectional flux may, in fact, appear to be unidirectional using the low-affinity lanthanum-resistant pool method, as the direction of ^{45}Ca flux would be overwhelmingly inward at early stages. Thus, the observed increase in influx provides two alternatives: either a true increase in intracellular calcium is occurring, or a rapid bidirectional flux is taking place. In the latter case, such a phenomenon can be seen with phospholipase C activation, whereby a high calcium influx may initially occur, followed by a coexisting high rate of efflux due to calcium pump activation. In support of this possibility, one should note that activation of protein kinase C via this pathway may result in delayed vascular smooth muscle contraction, with maximal contractions occurring at 45 to 60 minutes (29). In addition, activation of this pathway can lead to receptor "cross-talk" and may thereby provide an explanation for pressor potentiation. Indeed, increased phospholipase C activity has recently been noted in SHR vascular smooth muscle (30). Finally, one should also note that any apparent decrease in calcium uptake at 60 minutes in this study cannot be equated with a decrease in intracellular free calcium at this time point. Such a decrease in uptake may only indicate that a new intracellular-extracellular calcium equilibrium has been established. Thus, any decrease in calcium uptake may not necessarily explain or correlate with the eventual decrease in blood pressure.

In conclusion, this study confirms the ability of SHR plasma to increase blood pressure, increase calcium uptake and potentiate the action of vasopressors in normotensive animals. The time course of these events suggests that a single factor may be

responsible. The existence of such a factor may, in turn, explain the high intracellular calcium levels seen in some forms of hypertension, as well as the increased sensitivity of some hypertensives to pressor agents. Finally, the mechanism of action of this factor may be by directly increasing intracellular calcium levels in vascular smooth muscle, or alternatively, it may act by stimulating the phospholipase C pathway. Whether this factor is present throughout the development and maintenance of hypertension in SHR rats is not known. Similarly, the effect of treatment on the action or expression of this factor has not been determined. Clearly, more work will be needed in order to identify and elucidate the mechanism of action of this hypertensive substance and to determine its pathophysiological significance.

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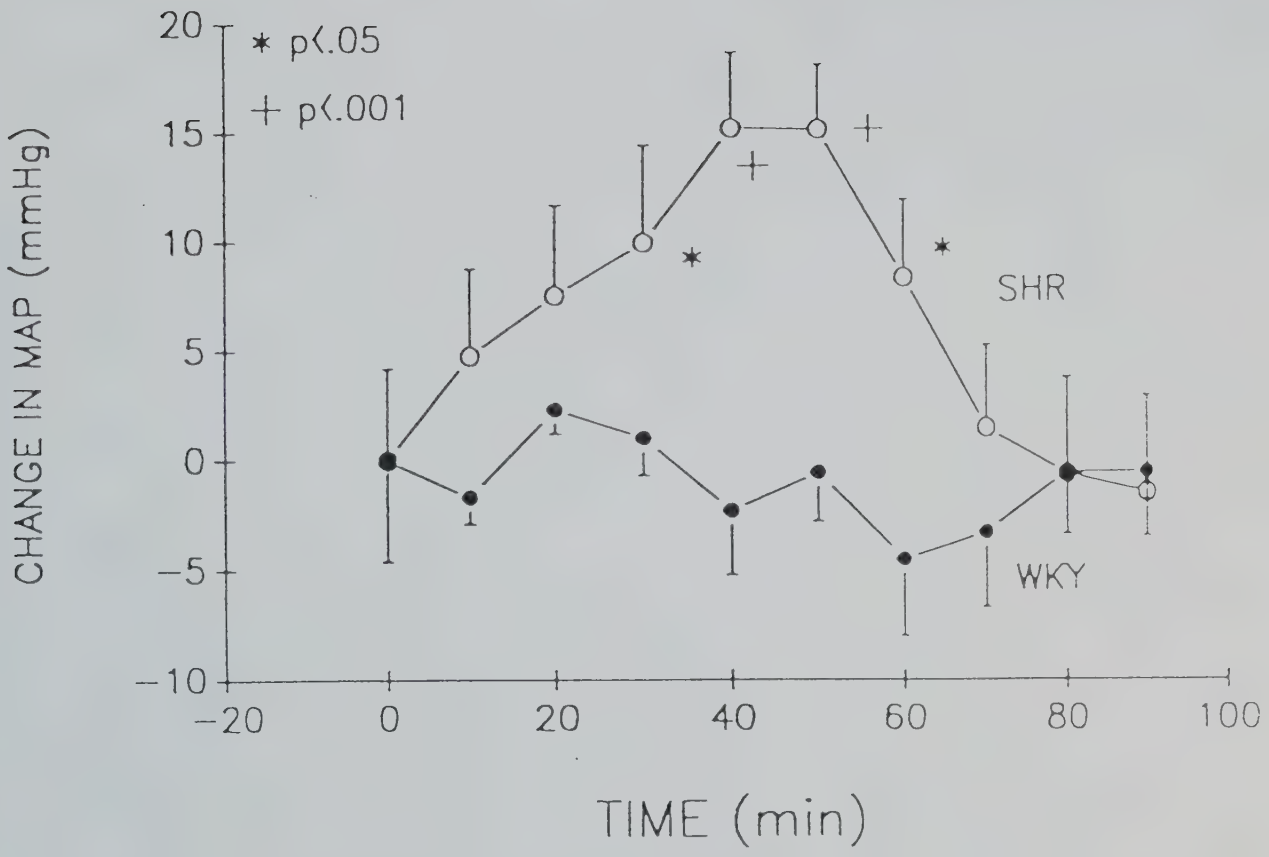

Figure II.1.

Effect of bolus administration of SHR and WKY plasma on blood pressure in normotensive SD rats. n=12 in each case. Means±SEM are given. *P* value is for difference from baseline pressure.

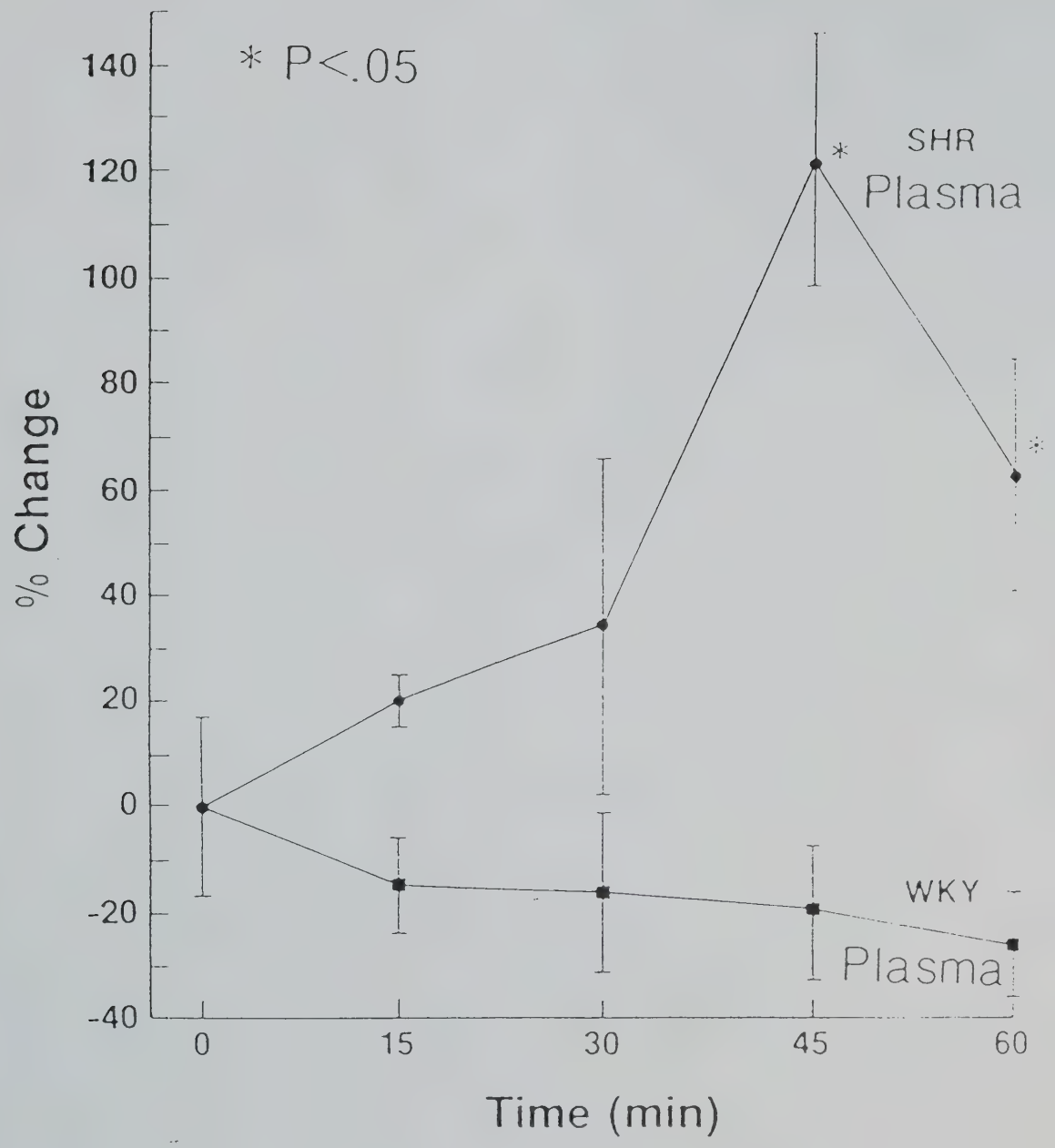

Figure II.2.

Effect of SHR and WKY plasma on the pressor response to norepinephrine. n=6 in each case. Means±SEM are given. *P* value is for difference from baseline response (time 0).

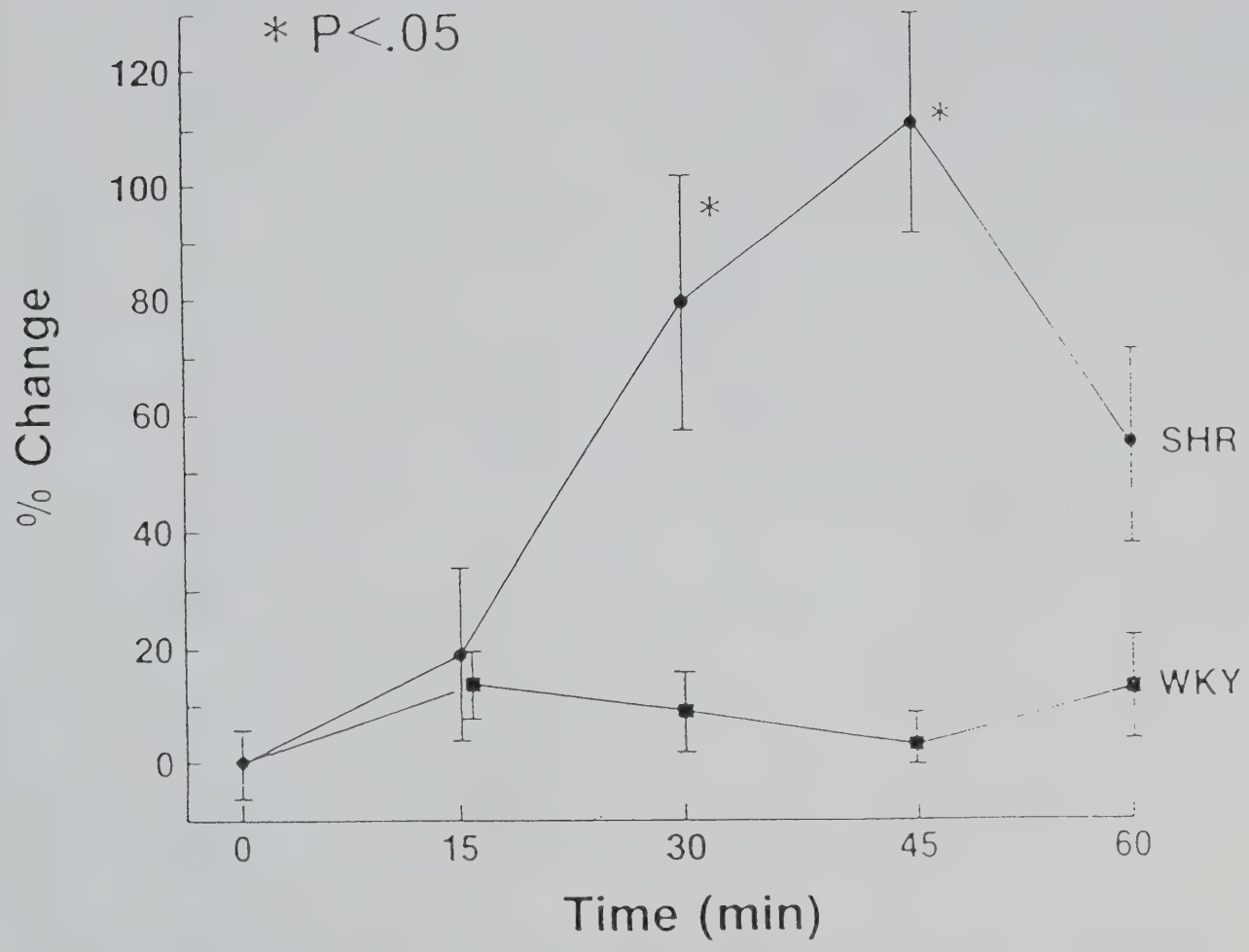

Figure II.3.

Effect of SHR and WKY plasma on the pressor response to angiotensin II. n=6 in each case. Means±SEM are given. *P* value is for difference from baseline response (time 0).

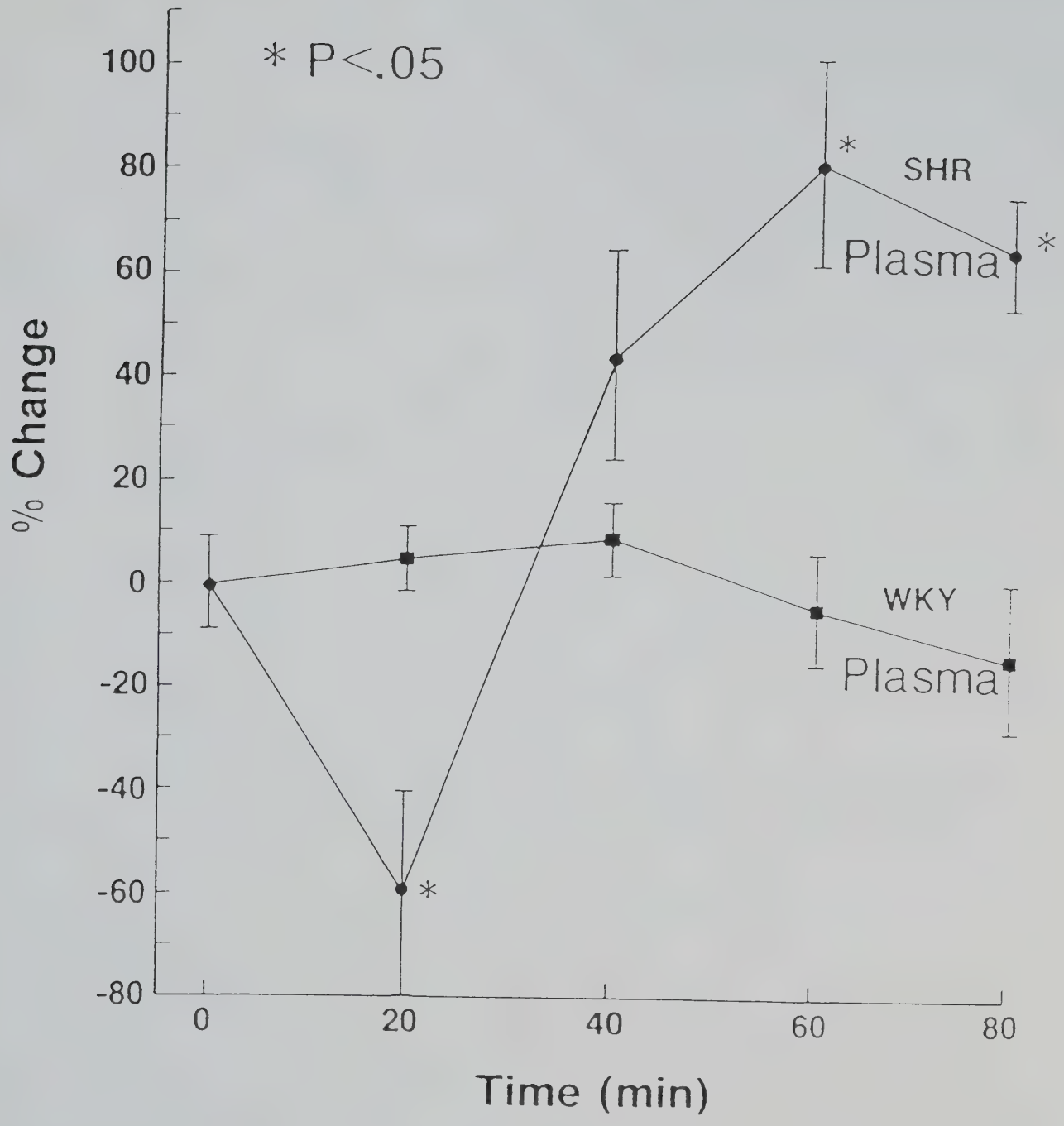

Figure II.4.

Effect of SHR and WKY plasma on the pressor response to arginine vasopressin. N=6 in each case. Means±SEM are given. *P* value is for difference from baseline response (time 0).

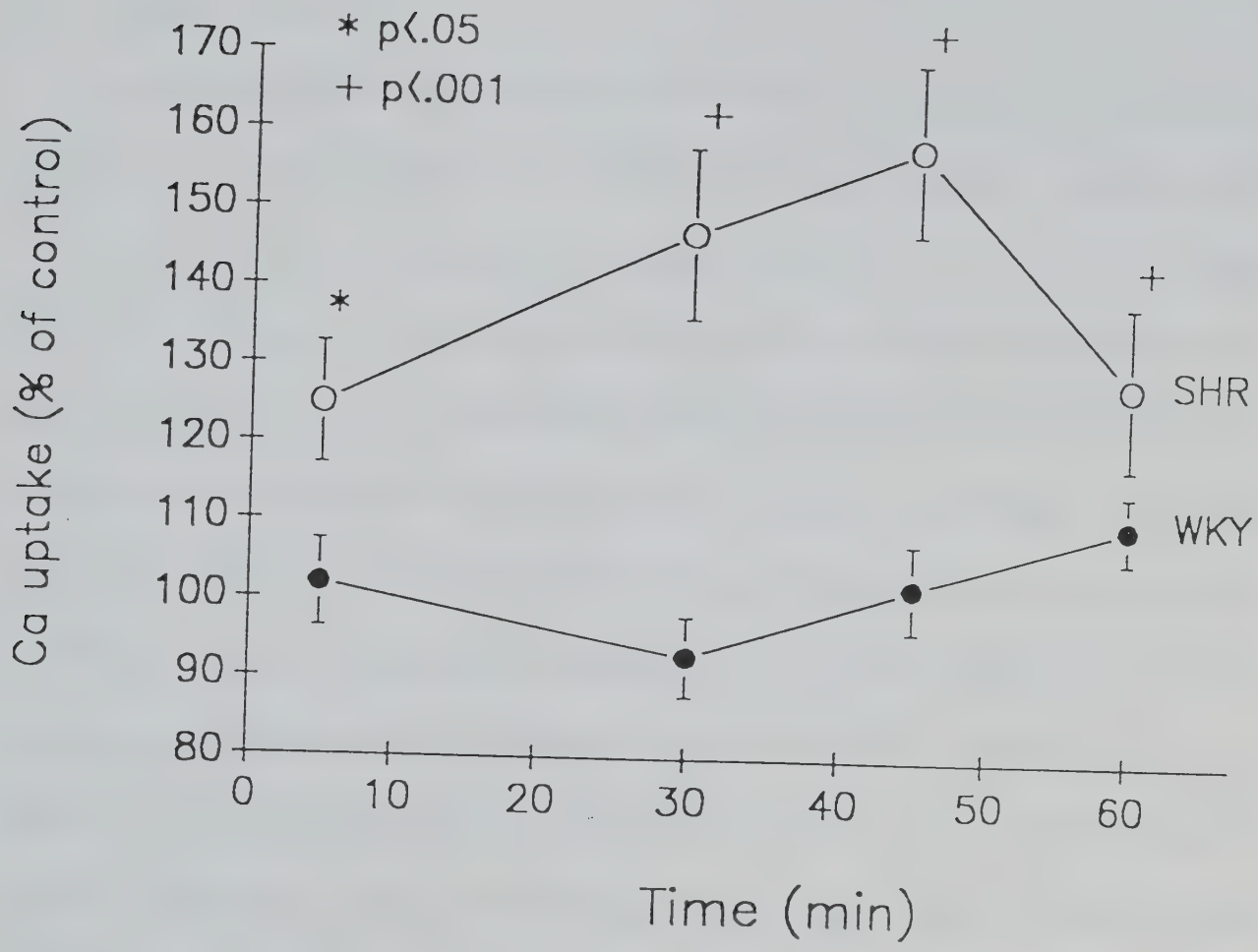

Figure II.5.

Time course for ^{45}Ca uptake in SD tail arteries in the presence of SHR and WKY plasma. n = 12 at each time period for each strain. Means ± SEM are given. *P* values are for difference from control (WKY) uptakes at each time period.

III. EFFECT OF SHR PLASMA ON THE VASCULAR ACTIONS OF PARATHYROID HORMONE

A. INTRODUCTION

In certain forms of hypertension, as found in Spontaneously Hypertensive Rats (SHR) and low-renin essential hypertensive patients, a discrepancy appears to exist between immunoreactive parathyroid hormone levels and the expected end-organ effects of such levels. Specifically, although PTH levels are elevated in these circumstances, the bioactivity for a given level of PTH is less than expected. Examples of such discrepancies include PTH level and ionized calcium (1,2), PTH level and renal $1,25(OH)_2$ vitamin D synthesis (3), PTH levels and intestinal calcium absorption (4), and PTH level versus renal calcium excretion (5). On the basis of these apparent discrepancies, we speculated that Parathyroid Hypertensive Factor (PHF) might be responsible for inhibiting or blocking the actions of PTH. To study this hypothesis, we chose to examine the effects of SHR and Wistar-Kyoto (WKY) plasma on the depressor effect of PTH.

B. METHODS

In vivo Effects

Male Sprague-Dawley (SD) rats weighing 300 to 350 g were used for this study. Rats were anesthetized with pentobarbital (65 mg/kg intraperitoneally) and

a carotid artery and jugular vein cannulated for blood pressure recording and injection of samples. The depressor response to intravenous injection of bPTH-(1-34) at a dose of 1.5 μg/kg was then measured. SHR or WKY plasma, previously dialyzed at 1,000 molecular weight cut-off, was then injected at a dose of 2.5 ml/kg. Following this, bPTH-(1-34) boluses of 1.5 μg/kg were injected intravenously at 10 minute intervals for 70 minutes and the depressor effect monitored by continuous intra-arterial blood pressure recording.

Effect of SHR Plasma on Cyclic AMP Generation by PTH

The effect of dialyzed SHR and WKY plasma on PTH-stimulated cAMP formation in vascular smooth muscle was studied by a modification of the method of Bergmann *et al.* (6). Briefly, male SD rats weighing 300 to 400 g were sacrificed by pentobarbital overdose and the tail arteries removed. The arteries were cut into strips, rinsed and incubated in oxygenated (95% O_2, 5% CO_2) Krebs buffer at 37°C for 2 hours. Krebs buffer was as follows: NaCl, 117 mM; KCl, 5 mM; NaH_2PO_4, 1 mM; $NaHCO_3$, 27 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.25 mM, glucose, 11 mM. After 105 minutes of incubation, isobutylmethylxanthine (IBMX) (Sigma, St. Louis) was added to the incubation solution at a final concentration of 0.5 mM. Fifteen minutes after adding IBMX, tissues were transferred to tubes containing 1 μM bPTH-(1-34) in Krebs buffer, 1 μM bPTH-(1-34) + 30% SHR plasma in Krebs buffer, or Krebs buffer alone. All solutions also had IBMX added to a concentration of 0.5 mM. Artery strips were incubated in the above solutions for 30 minutes, removed, blotted,

then immediately frozen on dry ice and weighed. Strips were then homogenized in 1 M $HClO_4$, the homogenate centrifuged at 3,000 g for 10 minutes and the supernatant saved for cAMP analysis. Cyclic AMP was measured by commercial radioimmunoassay (Amersham International, Amersham, UK).

Statistics

Depressor responses to PTH were compared to the baseline response by repeated measures of analysis of variance. Comparisons between cAMP groups were carried out by ANOVA using the Student-Neuman-Keuls test for *post-hoc* analysis.

C. RESULTS

Effect of SHR Plasma on Depressor Response to PTH

Baseline response to 1.5 μg /kg of bPTH-(1-34) was -14$\pm$2 mm Hg. As seen in Figure III.1, SHR, but not WKY, plasma rapidly attenuated the depressor effect of PTH. This effect persisted until 70 minutes post-plasma injection, at which time the baseline depressor response was regained. WKY plasma did not alter the depressor effect to PTH at any time. Tachyphylaxis to the depressor effect of PTH was not observed as manifested by the consistent depressor effect in the presence of WKY plasma and by virtue of the fact that PTH effect in the presence of SHR plasma eventually returned to baseline, despite repetitive PTH doses.

Effect of SHR Plasma on PTH-Stimulated cAMP Generation in Vascular Smooth Muscle

Figure III.2 shows the effect of SHR plasma on PTH-stimulated cAMP generation in rat tail artery. Baseline cAMP content of the tissue was 2.87±.55 nmol/g wet weight/30 minutes. PTH significantly stimulated cAMP formation with mean cAMP levels of 5.81±.22 nmol/g/30 minutes. In the presence of 30% SHR plasma, PTH-stimulated cAMP generation was inhibited, with mean cAMP levels of 4.82±.24 nmol/g/30 minutes (P=.02 versus PTH alone). There was no inhibition of PTH-stimulated cAMP generation in the presence of WKY plasma (6.21±.32 nmol/g/30 minutes, P=.34 versus SHR).

D. DISCUSSION

In this study, PHF-containing dialyzed SHR plasma is seen to inhibit the depressor effect of PTH, as well as PTH-stimulated cAMP generation in vascular smooth muscle. Presumably, the former effect is a consequence of the latter effect, as the vasodilatory action of PTH is thought to involve activation of adenyl cyclase (7). Interestingly, the presumed effect of PHF on inhibiting PTH vascular action occurs early and persists -- in the *in vivo* study, for 60 minutes after injection. The hypertensive effect of PHF, however, begins at 30 minutes and peaks at 50 to 60 minutes. The discordance between the two processes suggests that the pressor mechanism of PHF is indeed complex, possibly occurring as the net result of the interaction of a number of factors. Nevertheless, the current study does suggest that PHF

may be responsible for inhibiting some of the action of PTH in some forms of hypertension. Both the extent and mechanism of such inhibition remain to be elucidated, but if such inhibition is seen in other tissues, this may offer an explanation for the discrepancy between PTH levels and PTH actions in some forms of hypertension.

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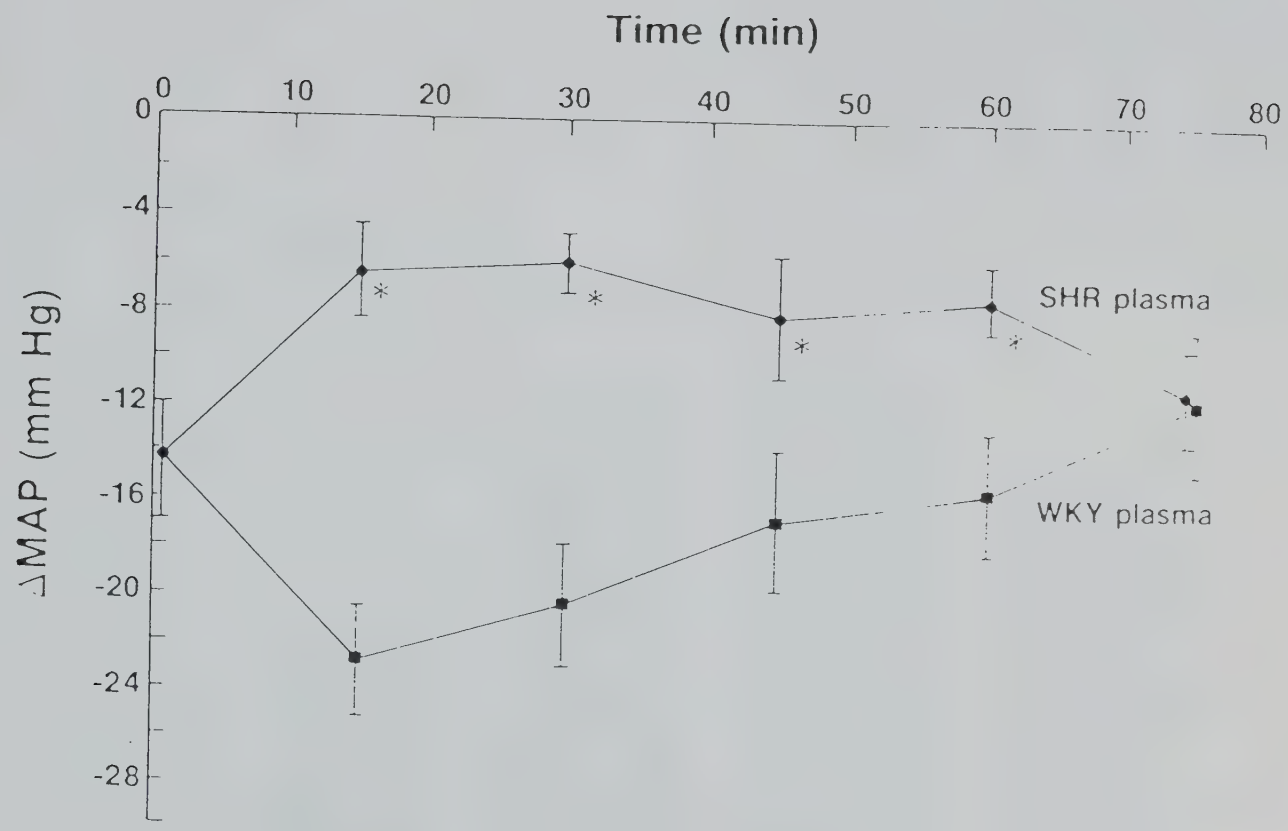

Figure III.1.

Effect of SHR and WKY plasma on depressor response to bPTH-(1-34). n=8 in each case. * denotes $P < .05$ versus time 0 by Student-Neuman-Keuls.

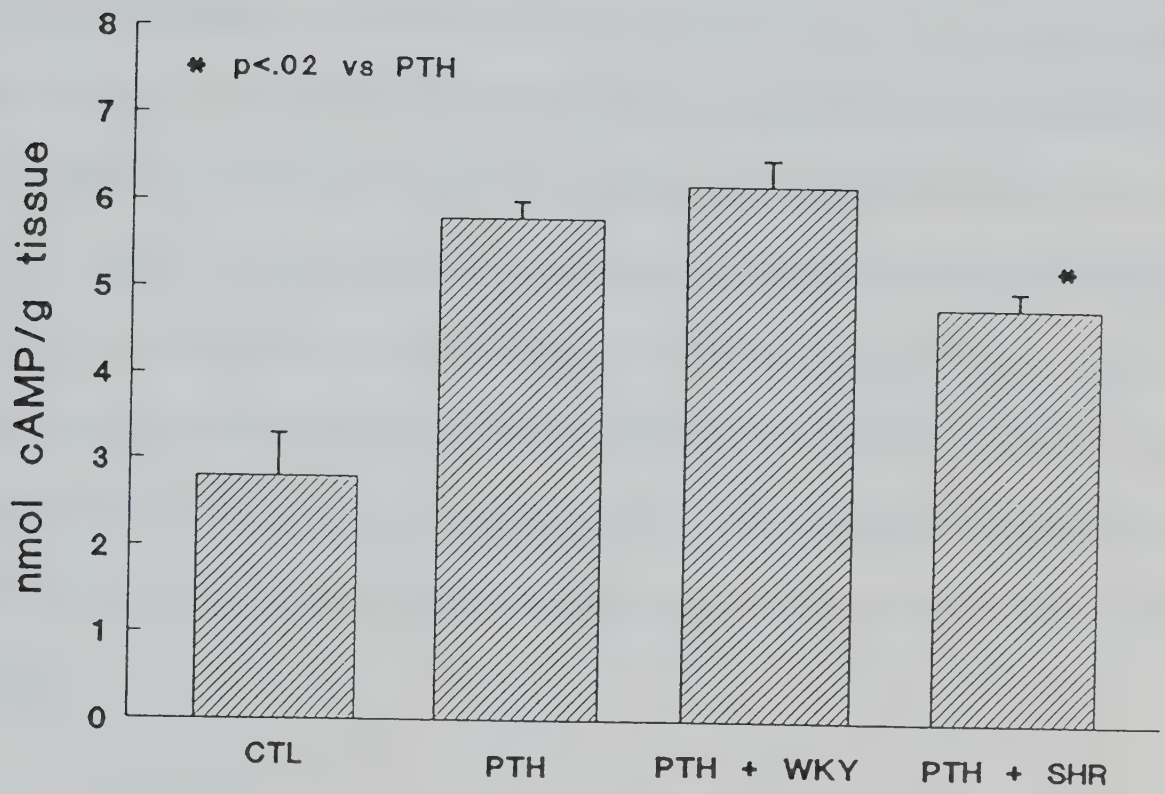

Figure III.2.

Effect of SHR and WKY plasma on PTH-stimulated cAMP generation in rat tail artery. Values given are for 30 minute incubations. n=12 in each case. * denotes $P<.02$ versus effect of PTH alone.

IV. EFFECT OF CALCIUM CHANNEL BLOCKADE ON THE PRESSOR EFFECTS OF PHF

A. INTRODUCTION

High intracellular calcium levels are seen in a variety of tissue types in Spontaneously Hypertensive Rats (SHR) (1-3). Parathyroid Hypertensive Factor (PHF) has previously been shown to increase calcium uptake into vascular smooth muscle (4) and to potentiate the action of certain calcium-dependent pressor agents (5) in SHR. On this basis, it might be hypothesized that PHF somehow acts on calcium channels to increase calcium uptake into vascular smooth muscle. This hypothesis is further supported by the observations that calcium channel blockers decrease blood pressure in SHR (6,7). Thus, we hypothesized that calcium channel blockers might antagonize the hypertensive effect of PHF. The purpose of this study was to determine whether calcium channel blockers could inhibit the hypertensive effects of PHF.

B. METHODS

Male Sprague-Dawley rats weighing 300 to 350 g were anesthetized (pentobarbital, 65 mg/kg intraperitoneally) and a carotid artery and jugular vein cannulated. Nifedipine (0.2 mg/kg), verapamil (1.0 mg/kg) or vehicle were then injected intravenously into the rats, followed by dialyzed (1,000 molecular weight cut-off)

SHR plasma at a dose of 4.5 ml/kg. Mean arterial pressure (MAP) was followed and the typical PHF effect quantitated as the characteristic sustained, delayed increase in MAP seen 30 to 60 minutes post-injection. PHF effects in the presence or absence of the calcium channel blockers were compared by paired *t* test using the null hypothesis that change in MAP should equal 0.

C. RESULTS

Effects of nifedipine and verapamil on the hypertensive effect of PHF are summarized in Figures IV.1 and IV.2, respectively. From these figures, one can see that both nifedipine and verapamil did block the pressor effect of PHF, whereas vehicle had no inhibitory effect on the PHF effect. In the case of both calcium channel blockers, MAP in the assay rats was initially decreased no longer than 10 minutes post-injection of the drug, with baseline pressures being regained prior to the observation of a PHF-like effect.

D. DISCUSSION

These experiments show that calcium channel blockers block the hypertensive effect of PHF. This suggests that, ultimately, PHF affects voltage-gated calcium channels, causing them to have either an increase in opening probability or opening duration. Whether the effect is direct or indirect remains to be elucidated. Given the delayed time course of the PHF effect, however, it is possible that the effect is indirect.

The results of this experiment corroborate the initial observations on the effect of PHF on ^{45}Ca uptake, whereby PHF was shown to increase calcium uptake into vascular smooth muscle (4). Since then, experiments utilizing the intracellular calcium-sensitive probe, Fura-2, have confirmed that PHF causes an increase in intracellular free calcium levels in vascular smooth muscle with a time course matching the effects of PHF on blood pressure and calcium uptake (8). On the basis of these findings and the current study, calcium channel blockers would seem to be efficacious in treating "PHF-positive" forms of hypertension. It is, therefore, of interest to note that calcium channel blockers are found to be most useful in low-renin forms of hypertension (9,10), where PHF levels are the highest (see Chapters XI and XII).

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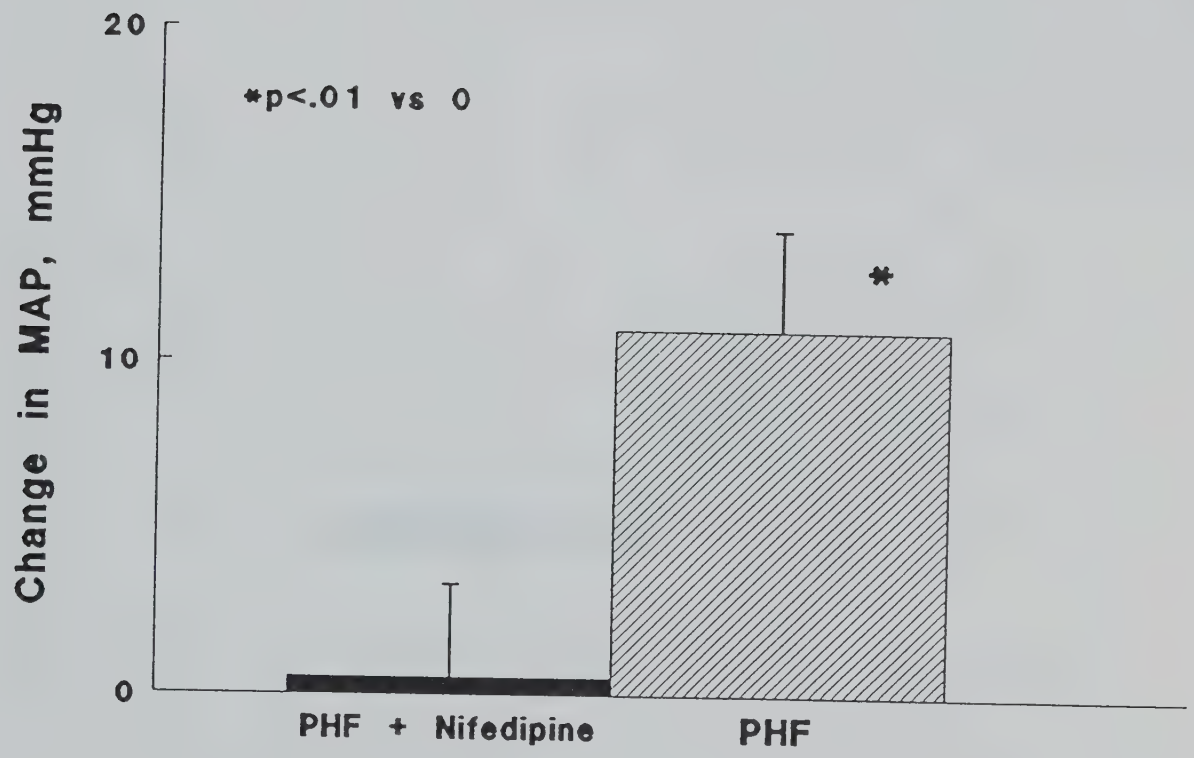

Figure IV.1.

Effect of nifedipine on pressor response to dialyzed SHR plasma. n=6 in each case. * denotes $P<.01$ versus null hypothesis that change in MAP should equal 0.

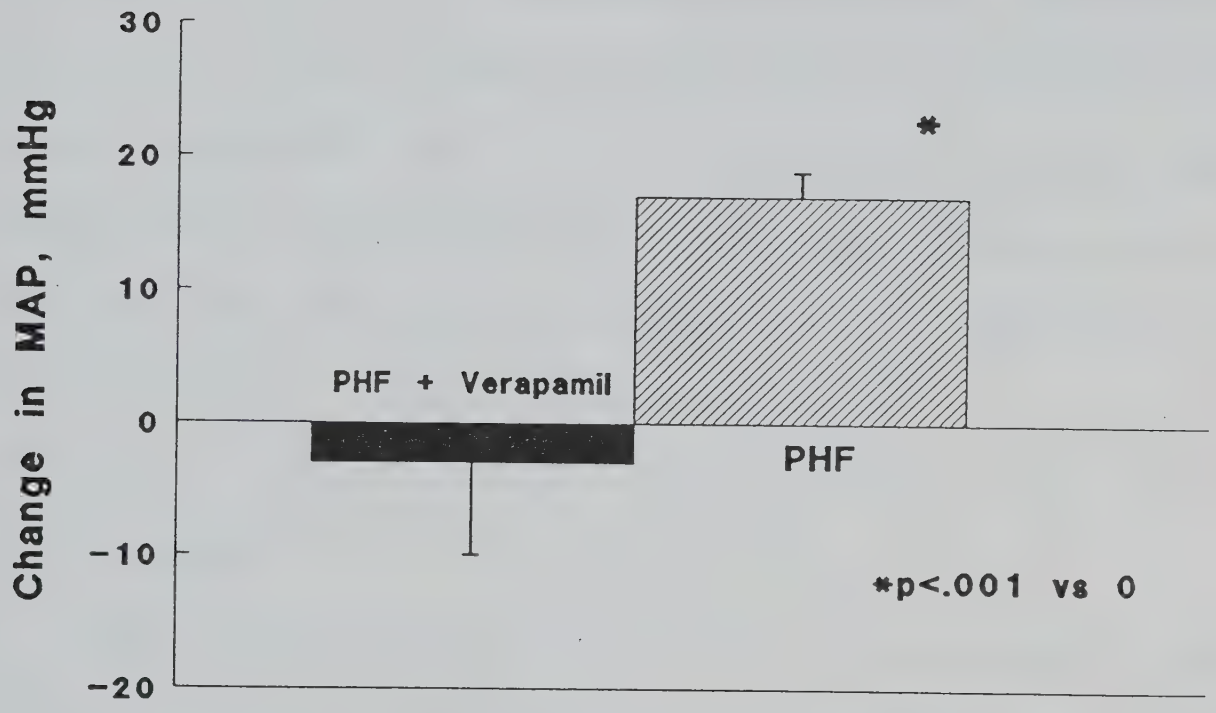

Figure IV.2.

Effect of verapamil on pressor response to SHR plasma. n=6 in each case. * denotes $P<.001$ versus null hypothesis that change in MAP should equal 0.

V. THE EFFECT OF PHF ON VASCULAR SMOOTH MUSCLE Na^+-K^+ ATPase

A. INTRODUCTION

For a number of years, circulating ouabain-like activity has been hypothesized to be involved in the genesis of hypertension via its inhibitory actions on Na^+-K^+ ATPase (1). Such inhibition, it has been reasoned, should result in an increase in vascular Na^+-Ca^{++} exchange in vascular smooth muscle, leading to vasoconstriction and an increase in blood pressure. The ouabain-like factor, therefore, represents a "circulating hypertensive factor". To ensure that Parathyroid Hypertensive Factor (PHF) -- another circulating hypertensive factor -- did not possess ouabain-like activity, the effect of PHF-containing SHR plasma on Na^+-K^+ ATPase activity was examined in the tissue of interest: vascular smooth muscle.

B. METHODS

Na^+K^+ ATPase activity was measured as the ouabain-sensitive ^{86}Rb uptake in rat tail artery using the method of Deth *et al.* (2). Briefly, male Sprague-Dawley rats weighing 300 to 400 g were sacrificed by pentobarbital overdose, and the tail arteries removed and cut into strips. The strips were rinsed in ice-cold Krebs solution (composition: NaCl, 117 mM; KCl, 5 mM; $NaHCO_3$, 27 mM, NaH_2PO_4, 1 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.25 mM; glucose 11.0 mM) and incubated in oxygenated (95% O_2, 5% CO_2) Krebs buffer at 37°C for 2 hours. For the final 30 minutes, Krebs

solution with no KCl and 122 mM NaCl was used to sodium-load the tissues. Following this incubation, the strips were transferred to tubes containing Krebs buffer and 1 μCi of ^{86}Rb, giving a final $RbCl_2$ concentration of 5 μM. Various incubation media consisted of the above-described incubation solution plus 30% SHR plasma (Rb concentration kept constant in all cases) plus 1 mM ouabain and incubation plus 30% plasma plus 1 mM ouabain. Strips were incubated in the respective solutions for 18 minutes, then rinsed in four changes of ice-cold Krebs solution, blotted, weighed and counted in a gamma counter. The effect of the various incubations on ^{86}Rb uptake was analyzed by ANOVA using the Student-Neuman-Keuls test for *post-hoc* comparisons. All results are expressed as mean$\pm$SD.

C. RESULTS

Results of this study are shown in Figure V.1. Baseline ^{86}Rb uptake was 2923$\pm$584 μmol/kg/minute. The ouabain-sensitive component (i.e. Na^+-K^+ ATPase activity) represented 46$\pm$18% of the total activity. In the presence of SHR plasma, ouabain-sensitive uptake was significantly increased to 181$\pm$68% of control ($P < .001$ versus control). When ouabain was added to the plasma, the effect of Na^+-K^+ ATPase was no different than in the presence of ouabain alone. This suggests that SHR plasma (and presumably PHF) stimulated Na^+-K^+ ATPase. WKY plasma had no effect on ouabain-sensitive ^{86}Rb uptake (results not shown).

D. DISCUSSION

From the results of this experiment, PHF would seem to have effects opposite to those of the ouabain-like factor -- stimulating, rather than inhibiting, Na^+-K^+ ATPase. This effect is in keeping with observations that SHR rats display increased Na^+-K^+ ATPase activity in their tissues (3,4). Admittedly, the effect being measured in this experiment is the effect of dialyzed SHR plasma on Na^+-K^+ ATPase, rather than the effect of pure PHF. Nevertheless, as the same plasma shows *in vivo* hypertensive effects, as well as stimulating effects of ^{45}Ca uptake in vascular smooth muscle *in vitro*, it is probably not unreasonable to assume that any ouabain-like activity would not be masked in the present system. That is, if Na^+-K^+ ATPase stimulatory activity is manifest (i.e. predominates) in plasma that can also increase blood pressure and vascular smooth muscle calcium uptake, it is unlikely that the latter effects are due to Na^+-K^+ ATPase inhibition. Thus, PHF is unique from the ouabain-like factor and may therefore represent a novel circulating hypertensive factor.

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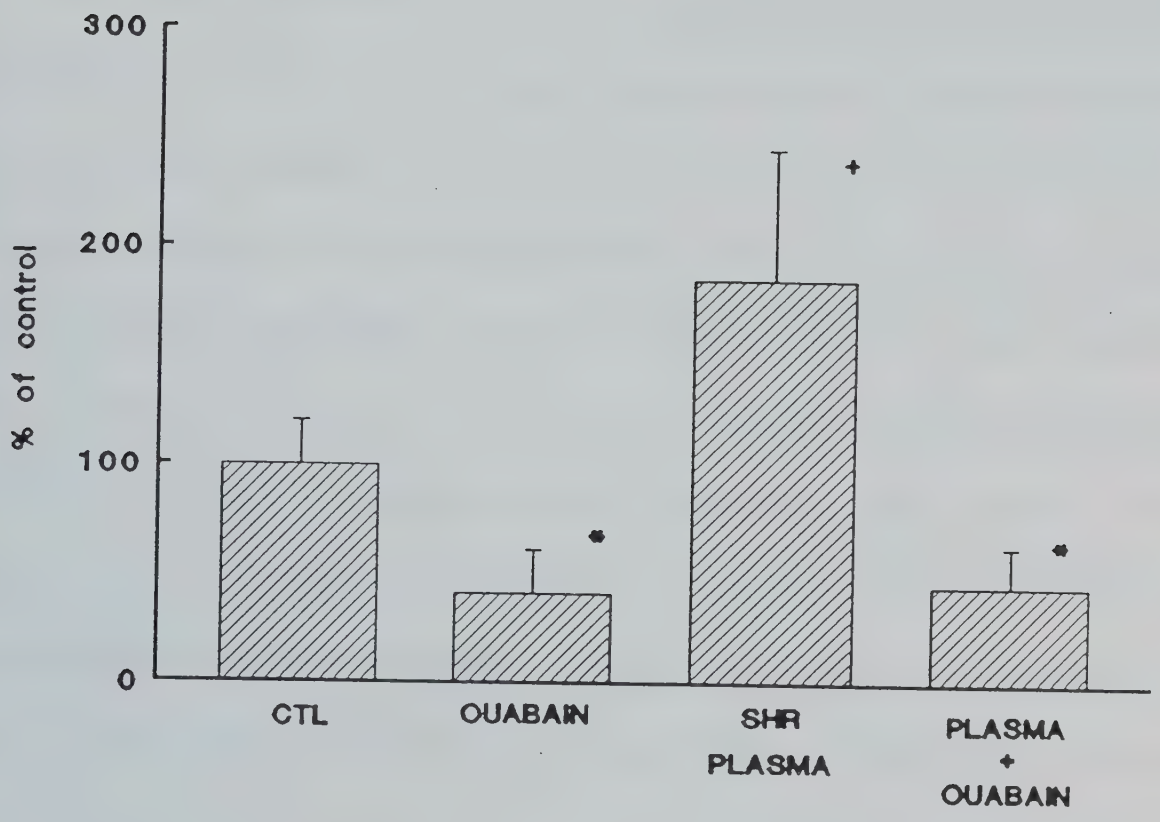

Figure V.1.

^{86}Rb uptake in the rat tail artery in the presence of ouabain and/or SHR plasma. n=8 for control (CTL), n=11 for ouabain. * denotes $P<.0001$ versus control ^{86}Rb uptake. + denotes $P<.001$ versus control ^{86}Rb uptake.

VI. THE EFFECTS OF DIETARY CALCIUM ON BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS MAY BE MEDIATED BY PARATHYROID HYPERTENSIVE FACTOR

A. INTRODUCTION

It has been recognized for a number of years that dietary calcium may affect blood pressure. This realization came as a result of studies in which an epidemiological association was noted between a low calcium intake and hypertension (1-3). Subsequent studies have directly examined the effects of a high calcium intake on blood pressure in hypertensive patients. Of these studies, some have suggested a beneficial effect of increased dietary calcium (4-6), while others have shown no effect (7-9). Thus, the role of dietary calcium in hypertension has remained unclear. Recently, it has been noted that a favorable response to a high calcium diet might be dependent upon a number of specific patient characteristics (10). These characteristics include older age, high serum parathyroid hormone (PTH) levels and a lower serum calcium.

Spontaneously hypertensive rats (SHR), which are often used as a model of

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human essential hypertension, are also sensitive to the antihypertensive effects of increased dietary calcium (11-13). Moreover, when placed on a high calcium diet, these rats show a decreased sensitivity to pressor agents (14). As with human responders, SHR also have increased PTH and decreased serum calcium levels (12). Recently, we have described the presence of a novel circulating hypertensive factor in the plasma of SHR (15). This factor increases blood pressure and vascular smooth muscle calcium uptake when given to normotensive rats. Additionally, it potentiates the actions of various vasopressors (16). This factor seems to be parathyroid-dependent but is not PTH. It has thus been termed "Parathyroid Hypertensive Factor", or "PHF" (17). If PHF is of parathyroid origin, then it is possible that it may also be under the same calcium-dependent regulation as PTH. Noting the antihypertensive effects of a high calcium intake in SHR, both on blood pressure and on vascular reactivity, and noting that a high calcium diet should suppress parathyroid gland activity, we hypothesized that this novel circulating hypertensive factor, PHF, might also be affected by altered calcium intake. The purpose of this study, therefore, was to observe the effects of high and low calcium intakes on the expression of this circulating hypertensive factor in SHR.

B. METHODS

Feeding

Four-week old male SHR were randomly assigned to one of three groups receiving a 0.02% (low), 0.6% (medium) or 2.0% (high) calcium diet as $CaCO_3$ by

weight. Diets were identical except for calcium composition. Rats were allowed free access to distilled water for the course of the experiment. Twenty rats were assigned to each group and ten rats were used as time 0 controls.

Blood Pressure and PHF Assay

At time 0, 4 and 8 weeks, random subgroups of rats were anesthetized (pentobarbital, 50 mg/kg intraperitoneally) and mean arterial pressure (MAP) directly measured via the right carotid artery. Plasma was then collected by decapitation, dialyzed at 1,000 molecular weight cut-off against saline at 4°C for 24 hours, and immediately assayed for PHF activity or stored at -90°C until used. Hypertensive factor activity was measured as previously described (15). Briefly, dialyzed plasma (2.5 mL/kg) was given as an intravenous bolus injection to pentobarbital-anesthetized Sprague-Dawley (SD) rats weighing 300 to 350 g. MAP was continuously monitored via a catheter in the right carotid artery and hypertensive factor activity was quantitated as the peak increase in blood pressure obtained 30 to 75 minutes post-injection.

Ionized Calcium and PTH Measurements

Ionized calcium was measured in freshly obtained plasma by a calcium-sensitive electrode (Ionetics, Costa Mesa, CA). PTH was measured by C-terminal radioimmunoassay (Diagnostic Systems Laboratories, Webster, TX).

Statistics

Differences in blood pressure within individual rats were assessed using Student's *t* test for paired data. Differences between groups of rats were assessed using analysis of variance and Student-Neuman-Keuls tests. The effects of various parameters on MAP in the rats were evaluated by multivariate analysis. All values are expressed as the mean±SEM.

C. RESULTS

Table VI.1 shows body weight, ionized calcium and C-terminal PTH values of the various rat groups during the course of the experiment. Body weight significantly increased in all groups over the course of the experiment, but was less at 8 weeks in the low calcium group when compared to the medium or high calcium groups. This difference, however, was not statistically significant due to the variation of weights in the low calcium group. Ionized calcium levels were also lower in the low calcium group when compared to initial values or as compared to the respective medium and high calcium groups. Medium and high calcium groups did not show any change in ionized calcium from initial values, nor did the values differ between these two groups. In all groups, C-terminal PTH was similar.

Figure VI.1 shows the MAP in all rat groups over the course of the experiment. Initial MAP was 111±7 mm Hg. At 4 weeks, only the high calcium rats showed a significant change in MAP, that being a decrease in MAP (95±4 mm Hg, $P < .05$). This decrease also resulted in a significant difference in MAP when compared to the

low calcium group. By 8 weeks, all three groups showed a significantly increased MAP as compared to the baseline values. The low calcium group at this point had a higher blood pressure than either the medium or high calcium groups. MAP in the high and medium calcium groups did not differ. Actual MAP values at 8 weeks for the low, medium and high calcium groups were 174±6 mm Hg, 145±5 mm Hg and 141±4 mm Hg, respectively.

Figure VI.2 shows PHF activity in plasma from the various groups. Significant PHF activity was noted in the low calcium rats at 4 weeks and in both the low and medium calcium groups at 8 weeks. This activity was greater in the low calcium group at 8 weeks than in the medium calcium group. The high calcium group did not show any PHF activity in their plasma at any time.

At 8 weeks, multivariate analysis showed that C-terminal PTH and ionized calcium had no effect on MAP, either overall or within any group. In contrast, PHF activity showed a strong positive correlation, both overall ($r=.78$, $P=.0001$) and within the low calcium group (r-.88, $P=.009$). PHF activity weakly correlated with MAP in the medium calcium group ($r=.70$, $P=.08$) and did not correlate at all in the high calcium group ($r=.25$, $P=.59$). Figure VI.3 shows the relationship between MAP and PHF activity for the three groups.

D. DISCUSSION

This study confirms the effects of dietary calcium on blood pressure in SHR rats. Specifically, a low calcium intake increases, and a high calcium intake attenu-

ates, the degree of hypertension in such animals. Similar associations and effects have been noted in some (4-6), but not all (7-9), human hypertensives.

At present, no explanations for the effects of dietary calcium on blood pressure have been found. In fact, the beneficial effects of a high calcium intake, and the deleterious effects of a low calcium intake, may seem somewhat paradoxical, given the increased levels of intracellular calcium associated with hypertensive animals (18-20) and humans (19,21,22). The current study, however, offers an explanation for this paradox. In rats on a high calcium diet, the secretion or production of the previously described hypertensive factor, PHF, is seemingly inhibited. As this factor stimulates calcium uptake into vascular smooth muscle (15), inhibition of PHF would be expected to decrease vascular tone, and hence blood pressure. Similarly, vascular sensitivity to calcium-dependent pressor agents would also be decreased, as has been reported (14). In contrast, a low calcium diet seems to stimulate the production of PHF, thus increasing blood pressure.

Currently, the only known substance which is stimulated by calcium deficiency and inhibited by calcium excess is PTH. The results of this study would seemingly rule out PTH as the putative hypertensive factor, as C-terminal PTH values were identical in all rat dietary groups, irrespective of PHF activity or blood pressure. This result is, perhaps, surprising, as PTH levels would have been expected to be high in the low calcium group, given their decreased ionized calcium levels. However, C-terminal PTH may not accurately reflect bioactive PTH. This may be due to an inhibition of the production or release of PTH by some mechanism in the presence of

the hypertensive factor. Alternatively, an increased proportion of intact N-terminal PTH may be present due to decreased degradation, as has been recently reported to occur in rats in states of calcium deficiency (23) and has been observed in Wistar-Kyoto rats in response to interventions which affect blood pressure (24). This still leaves the question, however, of how one can be sure that bioactive PTH is not the hypertensive factor.

PTH has been repeatedly shown to possess hypotensive properties (25-27). Moreover, evidence exists showing that PTH acts as an endogenous calcium channel blocker on vascular smooth muscle (28). Thus, the actions of PTH are opposite to those of PHF. In addition, in a recent study, we have shown that N-terminal PTH does not mimic the effect of PHF, nor does blood pressure correlate with C-terminal PTH under conditions of eucalcemia (17). In addition, C-terminal, mid-molecule and intact PTH values did not correlate with PHF activity or blood pressure in a group of hyperparathyroid patients (unpublished observations). Thus, the factor is different from PTH.

In the current study, the rats on the high calcium diet still exhibited an increase in blood pressure to hypertensive levels over time, although the magnitude of the increase was the least of the three groups. This is in spite of having no detectable PHF activity in their plasma. This suggests that PHF is not the sole cause of hypertension in SHR. This finding is in keeping with other studies showing that a high calcium diet attenuates, but does not prevent, the development of hypertension in SHR (11-13). Thus, a certain component of the hypertension in SHR may be due

to PHF, while the remainder may be due to other components.

Since altered calcium intake can also affect blood pressure in some human hypertensives, the question arises whether the effects of dietary calcium in human essential hypertension might also be mediated through changes in PHF. Indeed, PHF activity has been found in a proportion of human essential hypertensive patients (29). If this hypertensive factor does play a role in human hypertension, then the diversity of responses to dietary calcium in essential hypertensives may be explained. Specifically, those hypertensives whose high blood pressure is contributed to significantly by the hypertensive factor would be expected to respond favorably to a high calcium diet. Thus, it may be important to detect and quantitate the presence of this factor in human hypertensive patients.

In summary, the changes induced in blood pressure in SHR rats by dietary calcium manipulation may be mediated by changes in a novel circulating hypertensive factor. If this is the case, measurement of this factor may be a predictor of a favorable response to calcium supplementation in hypertension. Clearly, more work will need to be done in this area.

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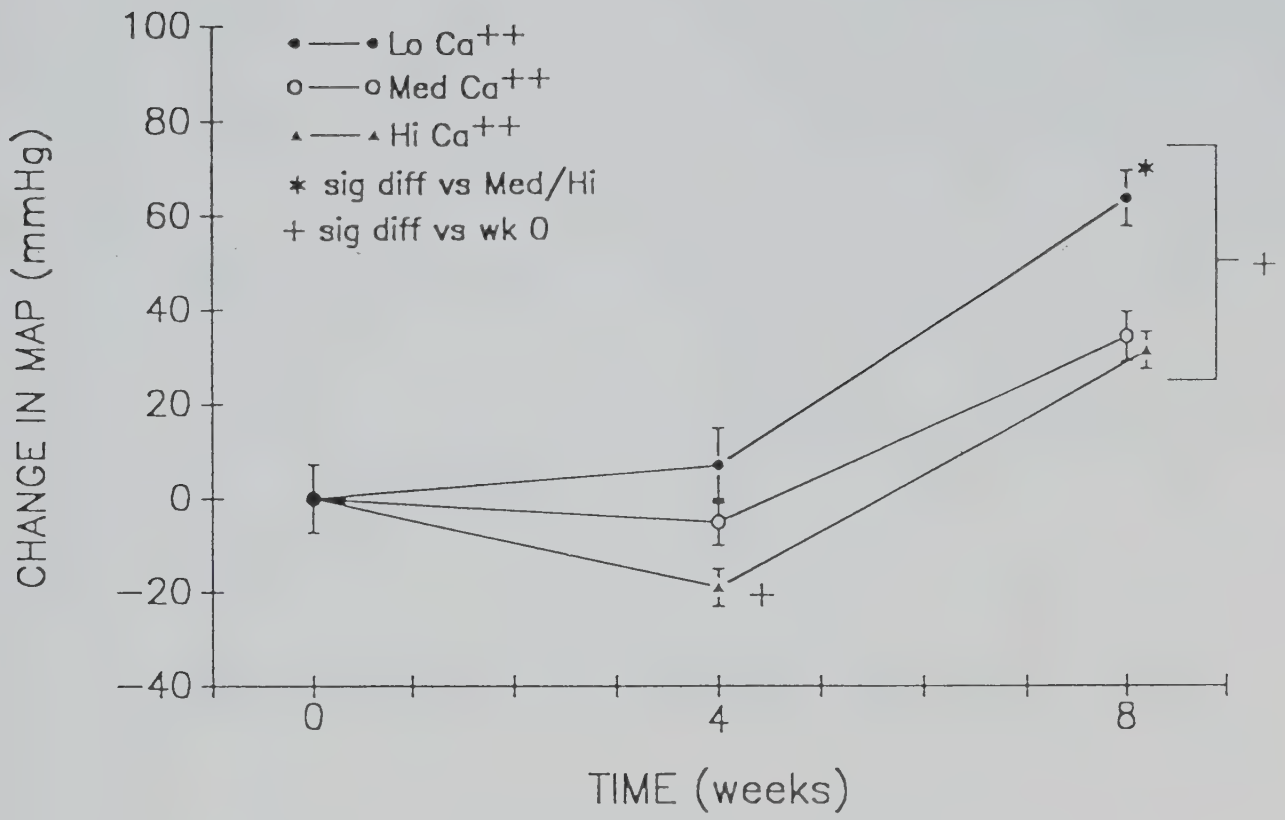

Figure VI.1.

Changes in blood pressure of SHR rats on various calcium content diets. $*P<.01$ versus Med/High, $+P<.01$ versus time 0, n=10 at each point.

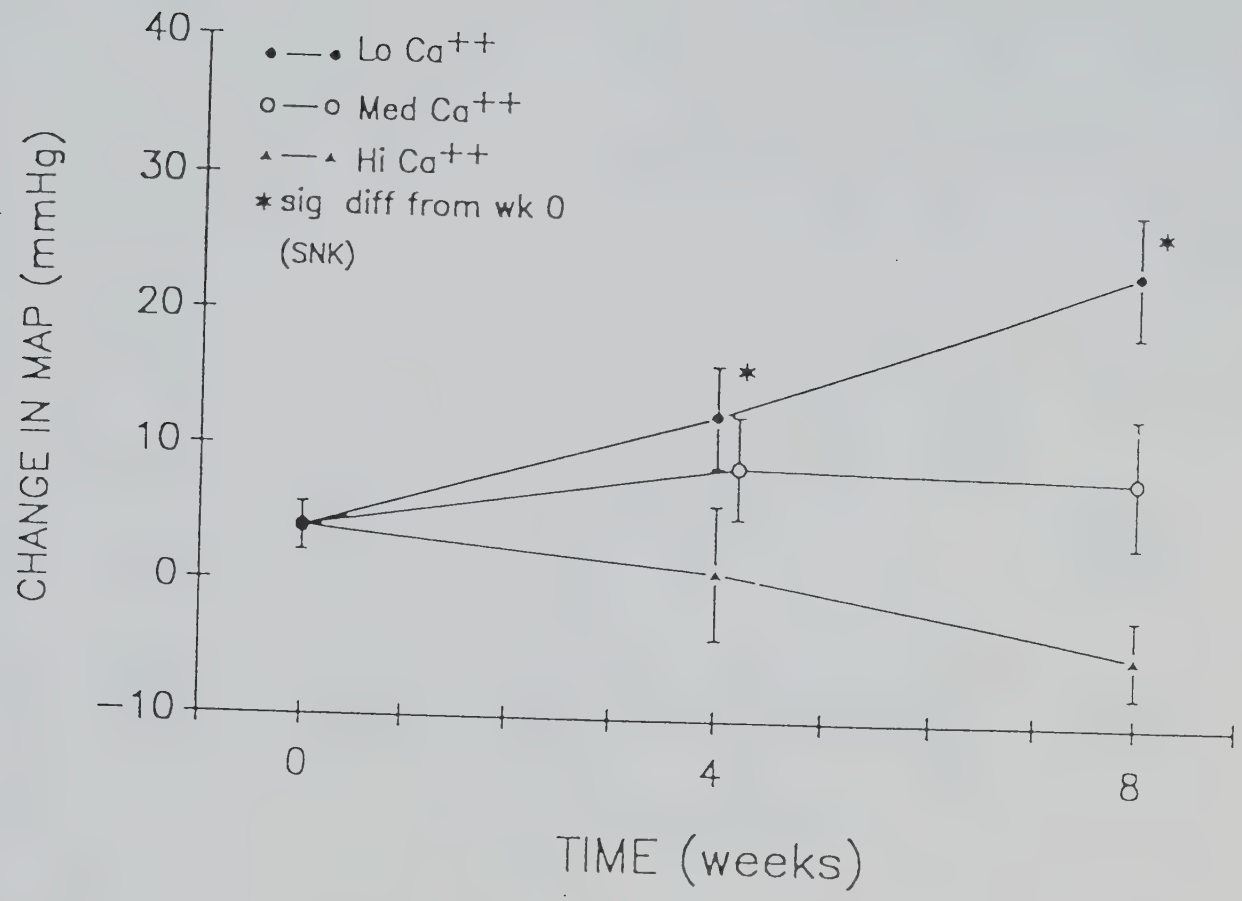

Figure VI.2.

PHF activity, given as change in MAP, in plasma from SHR rats on various calcium content diets. *$P < .05$ versus time 0 PHF level. n=7 at each point.

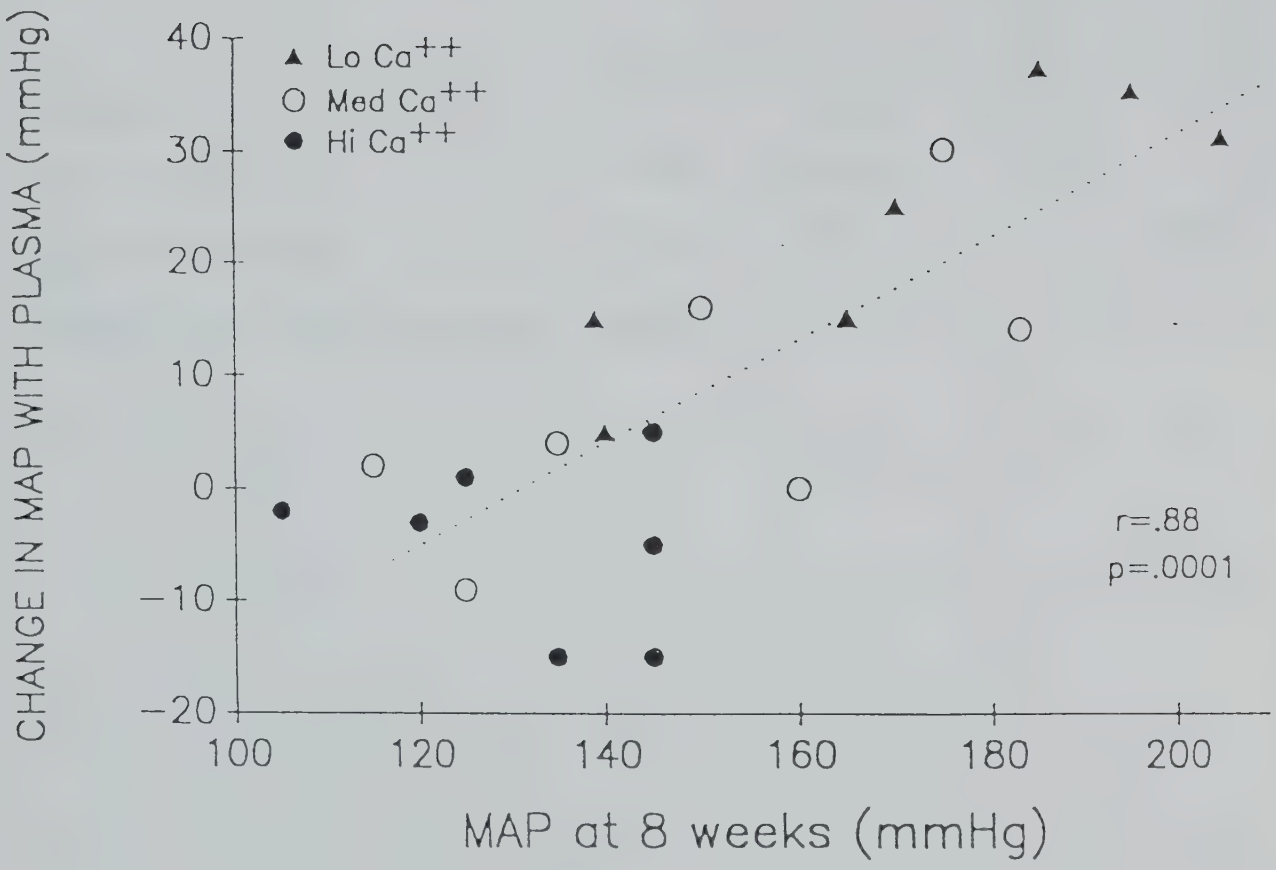

Figure VI.3.

Correlation between MAP and PHF activity. Overall correlation is shown on the graph (r=.78, $P<.001$). Within groups, the correlation coefficients and P values are as follows: Lo, r=.88, $P=.009$; Med, r=.70, $P=.08$; Hi, r=.25, $P=.59$.

Table VI.1.

Body weight, ionized calcium and C-terminal parathyroid hormone levels of SHR on various calcium diets.

		Week 8		
	Week 0	Low	Medium	High
Weight (g)	82	208±48	242±48	249±2
Ca^{++} (mmol/L)	1.2±.03	0.84±.02*	1.21±.01	1.24±.02
Parathyroid hormone (pg/mL)	361±26	364±21	367±11	360±25

*$P < .001$ versus week 0 and versus Medium/High. n=10 in each case.

VII. PARATHYROID ORIGIN OF A NEW CIRCULATING HYPERTENSIVE FACTOR IN SPONTANEOUSLY HYPERTENSIVE RATS

A. INTRODUCTION

Considerable evidence exists implicating the involvement of the parathyroid gland and its product, parathyroid hormone (PTH), in the development of some forms of human and experimental hypertension. In spontaneously hypertensive rats (SHR), parathyroidectomy significantly attenuates the development of hypertension (1-4), decreases hyper-responsiveness to pressor agents (5) and inhibits the hypertensive response to infused calcium (6). In deoxycorticosterone-induced hypertension in rats, parathyroidectomy has also been reported to inhibit the development of hypertension (7) and attenuate vascular damage and calcium accumulation in the heart, kidney and blood vessels (8,9). In both SHR (10,11) and deoxycorticosterone-induced hypertension (12), PTH levels have been found to be increased when compared to normotensive controls. Similarly, subsets of human essential hypertensive patients have also been found to have elevated PTH levels (13-16). Although it is obviously not known what the effects of parathyroidectomy are on blood pressure in

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human essential hypertension, it is known that primary hyperparathyroid patients are frequently hypertensive (17-19) and that parathyroidectomy often normalizes their blood pressure (20,21).

On the basis of the preceding studies, it would seem that PTH may play a causative role in the development of some forms of hypertension. Direct studies on the action of PTH on the vasculature, however, have shown that PTH causes vasodilation and a decrease in blood pressure (22-25). Thus, a paradox exists wherein the parathyroid gland seems to be important for the development of hypertension, yet its only known active product, PTH, is hypotensive. Recently, we described the presence of a new circulating factor in the plasma of SHR (26). The presence of this factor was associated with the presence of a novel cell type in the parathyroid glands of SHR (27). The purpose of this study, therefore, was to determine whether the parathyroid gland might be the source of this circulating factor. If such were the case, the importance of the parathyroid gland in hypertension could be reconciled with the hypotensive actions of PTH.

B. METHODS

Hypertensive Factor Assay

Plasma was collected from test animals and assayed for hypertensive factor activity, as previously described (26). Briefly, blood was obtained by decapitation, heparinized (100 IU/mL) and centrifuged at 3,000 g at 4°C for 10 minutes. The resultant plasma was dialyzed with a 1,000 molecular weight cut-off against saline for

24 hours at 4°C. The dialyzed plasma was then either used immediately or stored at -90°C. For the hypertensive factor assay, plasma obtained as noted above was warmed to 37°C and given as a bolus intra-venous dose of 2.5 mg/kg to anesthetized (pentobarbital, 50 mg/kg intraperitoneally) Sprague-Dawley (SD) rats weighing 300 to 350 g. Hypertensive factor activity was quantitated as the typical peak increase in mean arterial pressure (MAP) occurring 30 to 75 minutes after injection.

Infusion Studies

Infusion studies were carried out in SD rats anesthetized as described above. Infusions of plasma were carried out at a rate of 3.5 mL/kg/hour. Both bPTH-(1-34) and bPTH-(3-34) (bovine parathyroid hormones) were infused at a dose of 2.5 nmol/kg/hour.

Parathyroidectomy

SHR and SD rats of approximately 20 weeks of age were anesthetized as above and underwent placement of an indwelling arterial cannula as well as bilateral parathyroidectomy or sham operation. The latter consisted of exposure of the parathyroid glands. After measurement of MAP, the intra-arterial cannula was capped and filled with heparinized saline (1,000 IU/mL). Post-operatively, 1% $CaCl_2$ was provided in the drinking water to prevent tetany. After 48 hours, the rats were re-anesthetized and MAP recorded. Blood was then collected and plasma prepared and assayed for hypertensive factor activity as described above.

Parathyroid Gland Transplantation

Twenty-week old SHR and SD rats were anesthetized and had MAP determinations carried out via the carotid artery. The artery was ligated following this measurement. The parathyroid glands were then identified and gently dissected away from surrounding thyroid tissue. The glands thus obtained were either discarded or immediately transplanted into another newly parathyroidectomized rat. Transplantation was carried out using a single gland with the capsule incised. The recipient site was within the strap muscles of the neck, as this site seems to result in the best survival of transplanted parathyroid glands in the rat (28,29). No immunosuppression was used. Following surgery, rats were allowed to recover and were provided with 1% $CaCl_2$ in their drinking water for 2 weeks post-operatively while the transplants were establishing. Ninety days post-operatively, rats were re-anesthetized, MAP measured intra-arterially via the remaining carotid artery, and plasma obtained and assayed for hypertensive factor activity as described above. The site of transplantation was excised and examined histologically to confirm gland survival. Plasma was also assayed for total calcium and C-terminal PTH levels to ensure gland survival. Total calcium was measured calorimetrically and C-terminal PTH was measured by radioimmunoassay (Diagnostic Systems Laboratories Inc., Webster, TX).

Statistics

Changes in MAP over time within the individual rats were assessed using Student's *t* test for paired data. Differences between groups of rats were assessed

using analysis of variance or unpaired *t* tests as appropriate. Survival rates of transplants were compared by chi-square analysis.

C. RESULTS

Plasma and PTH Infusions

SHR plasma increased, bPTH-(1-34) decreased, and bPTH-(3-34) had no effect on blood pressure in SD rats. From Figure VII.1, it can be seen that the maximum pressor or depressor effects of SHR plasma and bPTH-(1-34) are achieved by 90 minutes, after which time a plateau is maintained.

Parathyroidectomy

The overall preoperative MAP of the SHR rats was 182 ± 6 mm Hg and of the SD rats 111 ± 7 mm Hg. Following surgery, MAP was seen to have decreased in the parathyroidectomy group to 145 ± 11 mm Hg ($P < .001$), but did not significantly change in the sham group ($\chi = 181 \pm 5$ mm Hg, $P = \text{NS}$). Parathyroidectomy in the SD rats had no effect on blood pressure. The data are shown in Figure VII.2. Figure VII.3 shows the effect of bolus intravenous injection of plasma from sham and parathyroidectomized SHR into SD rats. Plasma from sham-operated rats significantly increased blood pressure, whereas plasma from parathyroidectomized rats did not increase blood pressure.

Parathyroid Transplantation

Initial MAPs of SD and SHR rats were 106±3 mm Hg and 174±8 mm Hg, respectively. Ninety day survival of rats in the three groups was 50% for SD to SD, 66% for SHR to SD, and 60% for SD to SHR (χ=.908, P=NS). All surviving rats showed evidence of transplant survival as manifested by normal levels of total serum calcium and PTH at the time of death. The vast majority of deaths in non-survivors occurred after 2 weeks, when $CaCl_2$ supplementation was discontinued. (Rats which developed signs of tetany were destroyed for humanitarian reasons.) Presumably, these rats were ones in which transplant establishment had not occurred or in which transplant rejection had occurred. Histological examination of the transplanted glands of survivors at the time of death showed no evidence of rejection. Table VII.1 lists total calcium and PTH values of the three groups at the time of death. No difference was noted in either of these parameters in any group. The effects of the transplantation of MAP in the rats is shown in Figure VII.4. Similarly, the effect of plasma obtained at the time of death from these rats on MAP in normotensive assay rats is also shown in this figure. SD to SD transplantation had no effect on MAP and did not result in appearance of the hypertensive factor in plasma. SD to SHR transplantation resulted in a significant drop in pressure and in the disappearance of the hypertensive factor from the plasma. SHR to SD transplantation had opposite effects compared to SD to SHR transplantation, with a significant increase in MAP and the appearance of the hypertensive factor in the plasma. Typical infusion profiles of SHR to SD and SD to SD plasma are shown in Figure VII.5. From this figure, one can see

that SHR to SD plasma also increases MAP when given as a continuous infusion, with a time course similar to SHR plasma (Figure VII.1). Substitution of saline as the infusate at 105 minutes resulted in decrease of MAP back to baseline, as has been observed with SHR plasma (26). SD to SD plasma had no significant effect on MAP.

D. DISCUSSION

The results of this study show that the parathyroid gland is important for the expression of hypertension in SHR. This importance is not due to the production of PTH, but seems to be due to the expression of a novel circulating hypertensive factor, recently described (26).

The exclusion of PTH as being the putative hypertensive factor noted in this and other studies is provided by a number of observations in this study. First, infusion of N-terminal PTH, which has been shown to be the vascular-active portion of the molecule (30), resulted in a decrease in blood pressure, as has been repeatedly observed (22-25). Although it has been suggested that the hypertensive actions of PTH might be due to a chronic effect, rather than an acute effect (3,4), SHR plasma is seen to increase blood pressure in one set of rats at a time when PTH is still decreasing blood pressure in a parallel set of rats. One might argue that the infused dose of PTH was too high in the current study, and that lesser concentrations, as found in SHR plasma, result in a pressor effect, whereas higher concentrations result in a depressor effect. Despite numerous studies on the vascular effects of PTH, this

phenomenon has never been reported to our knowledge. Moreover, if such is the case, blood pressure should have transiently increased during the early stages of the PTH infusion, when circulating concentrations would have been low. It has been suggested that N-terminal-deleted or other PTH fragments may be hypertensive (31). Indeed, C-terminal fragments have recently been shown to have osseous activity (32). This suggestion is not supported by the infusion or transplantation experimental data. Specifically, bPTH-(3-34) had no effect on MAP in infusion studies, and in transplantation studies, C-terminal PTH levels were identical in all rat groups, despite different blood pressures and different levels of hypertensive factor activity. Similarly, in hyperparathyroid human subjects, blood pressure and hypertensive factor activity show no relationship to intact PTH levels (unpublished data). Thus, it would seem that the hypertensive factor is not PTH or one of the usually occurring PTH fragments.

Although previous studies have shown that parathyroidectomy attenuates the development of hypertension in SHR (1-4), the reason for this phenomenon has been open to speculation. It has not been known whether the parathyroid gland has a permissive effect on the expression of hypertension, or whether PTH does, indeed, have a hypertensive effect. In the current study, parathyroidectomy did decrease blood pressure, but this decrease was also associated with a loss of hypertensive factor from the plasma. This effect would seem to be specific to the SHR gland, as parathyroidectomy in SD rats had no effect on blood pressure. The transplantation data provide further evidence for the unique role of the SHR parathyroid gland in the expression

of hypertension. This experiment showed that substitution of an SD parathyroid gland for the SHR's parathyroid glands resulted in a fall in blood pressure and in the inability to detect the factor in their plasma. Conversely, substitution of SHR parathyroid glands for SD parathyroid glands resulted in an increase in blood pressure in the SD rats and in the presence of the factor in their plasma. These effects, again, were specific to SHR parathyroid glands, as SD to SD transplantation had no effect. Similarly, the effects cannot be attributed to different calcium or PTH levels, as both parameters were similar in all transplant groups. It should be noted, however, that although MAP decreased in SD to SHR transplants, it did not normalize. Thus, the hypertensive factor may not be the sole cause of hypertension in SHR rats. Similar results were also noted for simple parathyroidectomy.

In the transplant experiments, one might wonder about confounding effects of cross-strain transplantation. This does not seem to have been a problem, as organ survival rates for SHR to SD and S to SHR were the same as for SD to SD. Similarly, the observed effects on blood pressure and hypertensive factor activity cannot be attributed to the cross-strain transplantation, as the results of transplantation were opposite in the former two cases.

In summary, the parathyroid gland of the SHR plays an important role in the expression of hypertension in these animals. This importance is due to the production or activation of a circulating hypertensive factor which is not PTH. Whether the factor is produced or activated by the gland is the subject of ongoing investigations. The current studies may also explain the cause of hypertension and the beneficial

effect of parathyroidectomy in hypertensive hyperparathyroid patients. In addition, given the abnormalities of parathyroid gland function in some human essential hypertensives, this factor may also be operative in these patients. In conclusion, given the importance of the parathyroid gland in the expression of the hypertensive factor, we have termed this factor "Parathyroid Hypertensive Factor", or "PHF".

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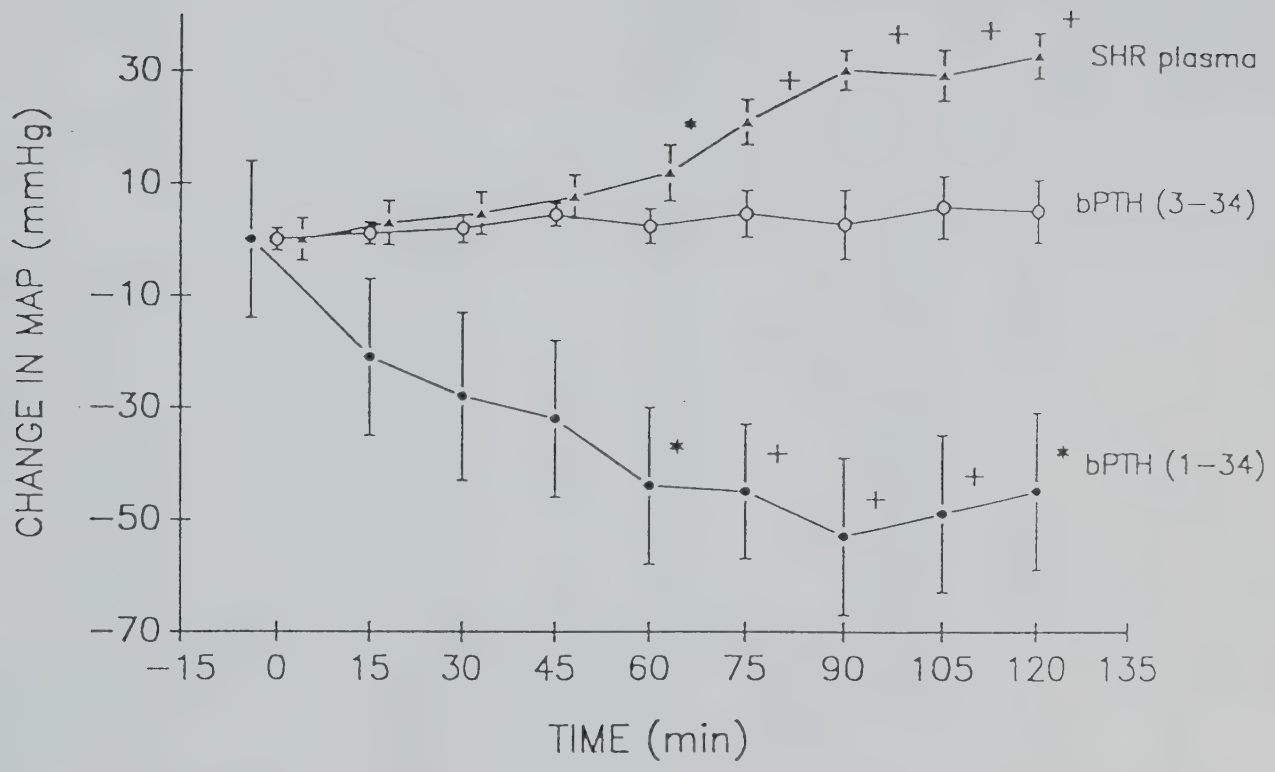

Figure VII.1.

Effect of infusion of bPTH-(1-34), bPTH-(3-34) and SHR plasma into normotensive rats. n=8 in each case. *$P<.05$, +$P<.01$ versus baseline pressure for individual rats.

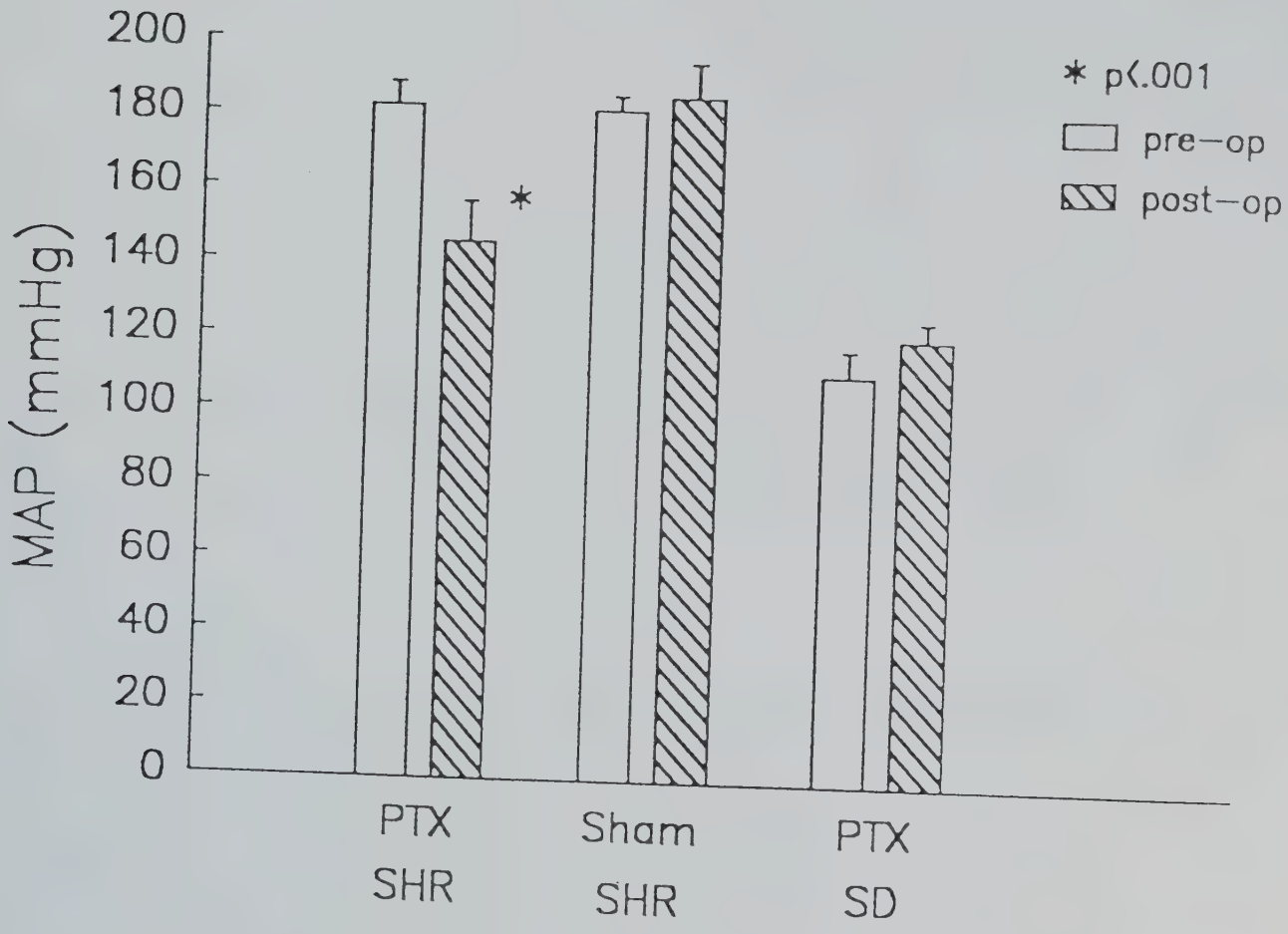

Figure VII.2.

Effect of parathyroidectomy (PTX) and sham operation (Sham) on MAP in SHR and SD rats. n=8 in each case. * denotes $P<.001$ compared to pre-operative MAP.

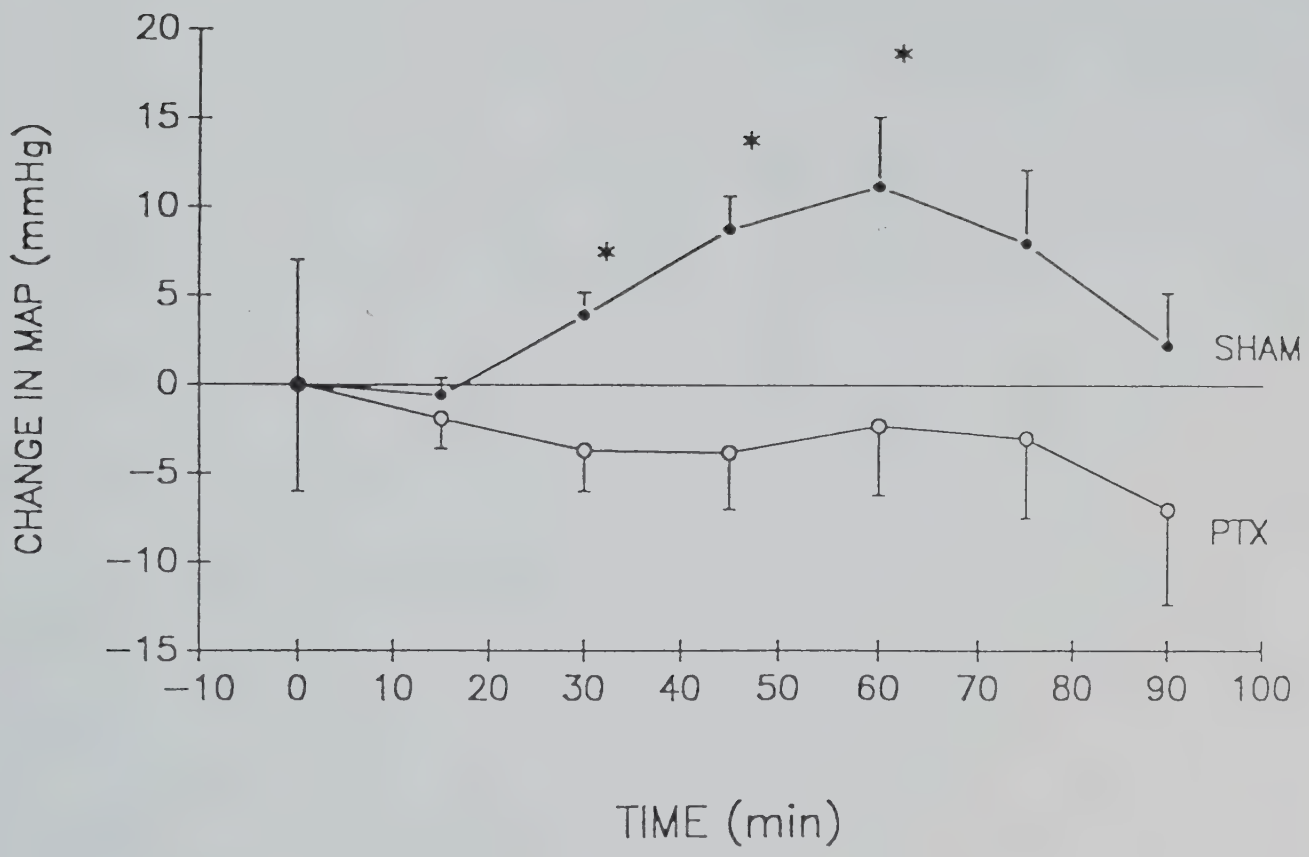

Figure VII.3.

Effect of bolus intravenous injection of plasma from parathyroidectomized (PTX) and sham-operated (SHAM) SHR on MAP in normotensive assay rats. n=8 in each case. $^{*}P<.01$ versus baseline pressure for individual rats.

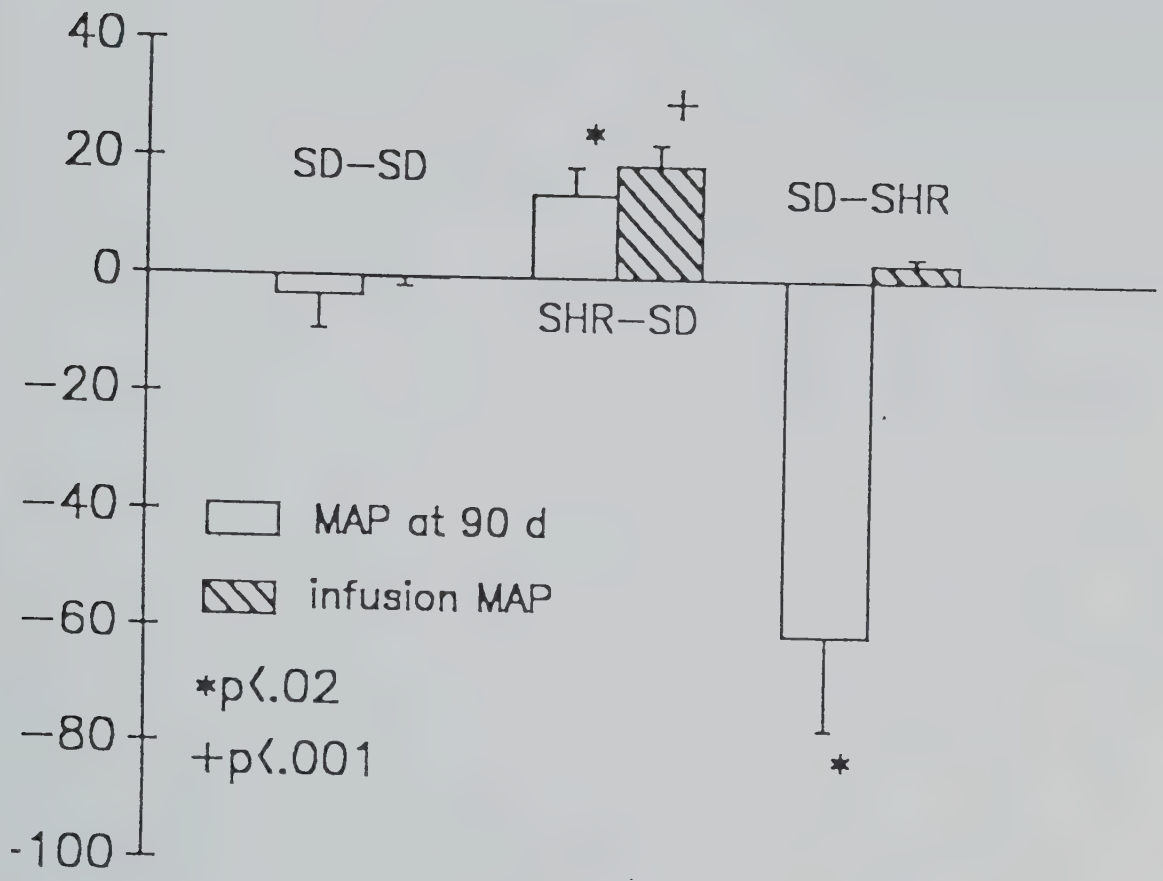

Figure VII.4.

Hypertensive factor activity and change in MAP 90 days following parathyroid gland transplantation. MAP at 90 days refers to the change in MAP of individual rats over time, and infusion MAP refers to the effect of infusing plasma from the same rats into normotensive assay rats. Transplants were from the first indicated strain into the second indicated strain. N = 10 for SD-SD, N = 15 for SHR-SD and n = 5 for SD-SHR. * denotes $P < .02$ versus initial MAP. + denotes $P < .001$ versus 0 for null hypothesis that change in MAP after plasma injection should equal 0.

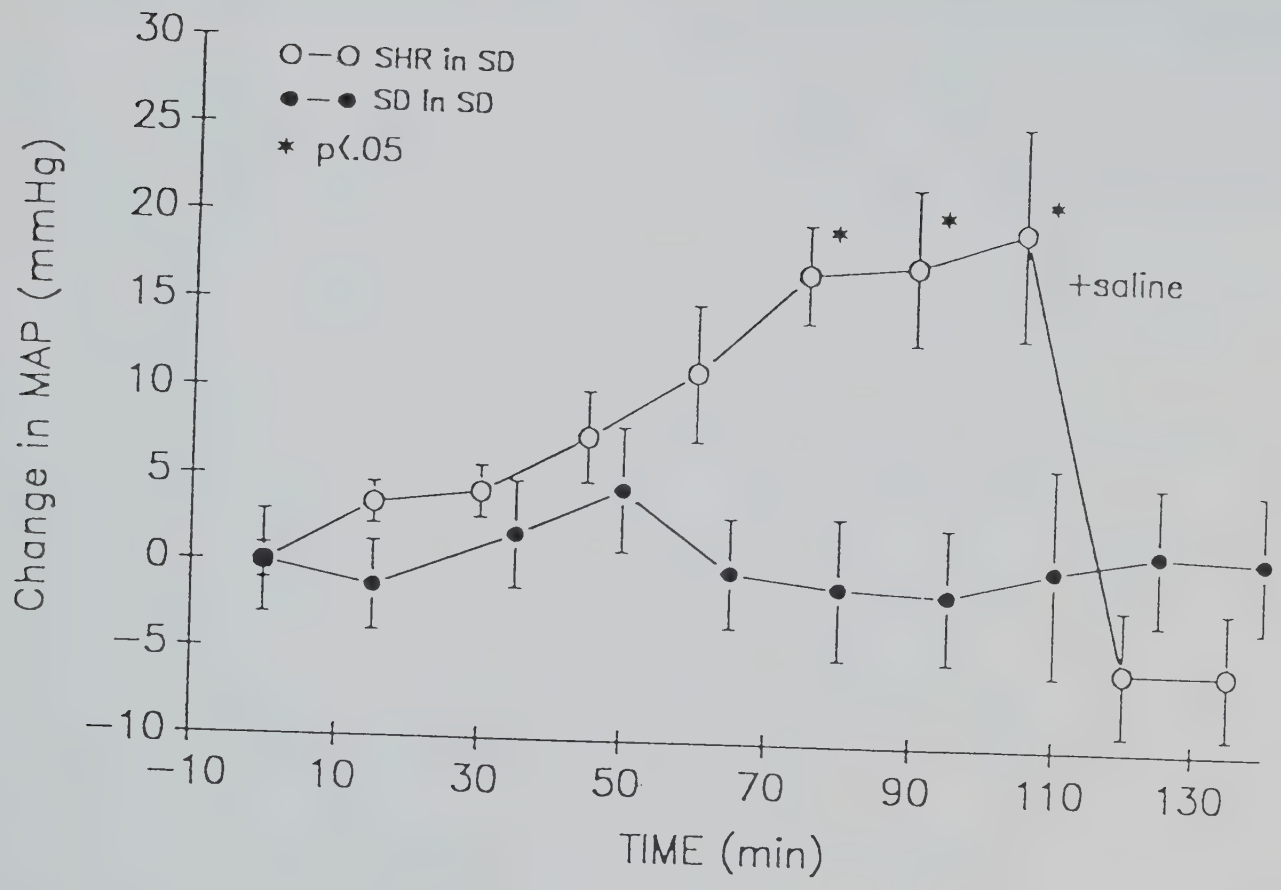

Figure VII.5.

Effect of infusion of plasma from transplanted rats into normotensive assay rats. At 100 minutes, saline was substituted for SHR-SD plasma in the infusion. n=6 in each case. * denotes $P<.05$ versus time 0. Transplants were from the first indicated strain into the second indicated strain.

Table VII.1.

C-terminal PTH and total calcium values in transplanted rats

	Transplant		
	SD-SD	SD-SHR	SHR-SD
C-terminal PTH (pg/ml)	390±64	330±64	356±25
Total serum calcium (mmol/L)	2.15±.17	2.18±.21	2.22±.2

VIII. A NEW CIRCULATING HYPERTENSIVE FACTOR IN THE PLASMA OF ESSENTIAL HYPERTENSIVE SUBJECTS

A. INTRODUCTION

The etiology of essential hypertension is unknown. Much clinical and laboratory experience suggests a heterogeneity of underlying factors, elevated blood pressure being a common manifestation of syndromes with different primary etiologies. Various reports have suggested the existence of as yet uncharacterized humoral factors in association with primary-genetic experimental as well as clinical essential hypertension (1-8). Our own group has recently reported the existence of a plasma hypertensive factor (9) in the plasma of spontaneously hypertensive rats (SHR), but not Wistar-Kyoto (WKY rats). This factor increases blood pressure when injected into the intact Sprague-Dawley (SD) rat, increases calcium uptake into rat tail artery strips, and appears to be of parathyroid origin, but is not identical with parathyroid hormone itself (10,11).

We report here a study in human subjects, demonstrating the presence of a circulating pressor factor with characteristics similar to those reported previously in

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SHR, among subjects with essential hypertension, but not in normotensive control subjects.

B. MATERIALS AND METHODS

All subjects were evaluated at the Cardiovascular Center of The New York Hospital, arriving after an overnight fast with a 24 hour urine specimen collected the previous day. Hypertensive patients were diagnosed as having essential hypertension on the basis of blood pressures $>150/90$ mm Hg measured on three separate occasions, in the absence of history, physical examination, or laboratory evidence of secondary forms of hypertensive disease.

No antihypertensive medications had been used for at least 3 weeks before the evaluation, and no diuretics had been used for at least 2 months previously. Normotensive subjects were recruited from a population followed annually by one of the present authors (LMR) as part of an epidemiologic, longitudinal study of the onset and incidence of hypertension in a stable population, and were well accustomed to the testing procedures used for hypertensive subjects. Blood pressure was measured in the seated position, and then blood was drawn for analysis of serum electrolytes, blood urea nitrogen, creatinine, plasma renin activity, and plasma hypertensive factor. Urine was analyzed for sodium excretion. Serum and urine electrolytes, blood urea nitrogen, and creatinine levels were analyzed by autoanalyzer techniques. Plasma renin activity was measured by standard radioimmunoassay techniques (12).

Blood collected for the analysis of plasma hypertensive factor was centrifuged

immediately after drawing and the plasma was decanted and frozen at -20°C, then transported on dry ice to the Department of Physiology, University of Alberta Medical School, by one of the authors. All patient samples arrived intact and frozen. They were then thawed and dialyzed for 24 hours at 4°C in spectropor dialysis tubing against normal saline. The molecular weight cut-off was 1,000. For the plasma hypertensive factor assay, dialyzed plasma (4 ml/kg) was injected intravenously into normotensive, pentobarbital-anesthetized (50 mg/kg intraperitoneally) SD rats weighing 300 to 350 g. Mean arterial pressure was continuously monitored for 90 minutes after the injection by means of a catheter in the right carotid artery. Plasma hypertensive factor was measured as the peak change in blood pressure induced in the SD rats over the 90 minute observation period. Blood pressure responses to hypertensive plasma samples were compared with those from normotensive control subjects. All samples were prepared and analyzed by personnel (RZL) unaware of the clinical status of the patients from whom the samples were obtained.

The effects of the plasma samples on ^{45}Ca uptake from normotensive and thirteen of the fourteen hypertensive patients in whom sufficient blood was available were determined by the low-affinity lanthanum-resistant pool method, as previously described (13,14). Briefly, de-endothelialized rat tail artery strips were equilibrated in oxygenated Kreb's buffer for 2 hours at 37°C, and were then incubated for 30 minutes in tubes containing ^{45}Ca and 30% plasma. The specific activity of the radiocalcium used was 24.6 mCi/mg, with a final specific activity in the bath of 0.3 μCi/ml. The strips were then rinsed in ice-cold, calcium-free lanthanum solution (50

mmol), weighed, digested overnight, and counted in a scintillation counter. The ratio of tissue to medium ^{45}Ca was used to determine calcium uptake values. The basal levels of calcium uptake, 2×15^5 counts/minute per gram tissue, were similar to those given in the original description of this technique (14).

All results are reported as means $\pm$ SEM. Two-tailed, unpaired Student's *t* tests were used to compare values obtained for all of the parameters measured, and linear regression with Pearson correlation coefficients was used to correlate the pressor effects and the calcium uptake stimulated by the plasma extracts.

C. RESULTS

Clinical and laboratory data obtained in the normotensive and hypertensive subjects studied are reported in Table VIII.1. The subjects did not differ with respect to age, sex or racial distribution, or renal function as indicated by blood urea nitrogen and creatinine values. Furthermore, average 24 hour urine sodium excretion values and plasma renin activity levels were similar in the two groups. However, aside from blood pressure itself, strikingly different levels of plasma hypertensive factor activity were found in the hypertensive versus the normotensive samples, the time-course of which is demonstrated in Figure VIII.1. While dialyzed normotensive plasma had little effect on blood pressure in the anesthetized rat bioassay model, and saline control injections of equal volume had no pressor activity (unpublished observations), the dialyzed hypertensive plasma demonstrated a consistent, significant pressor activity in the same assay system (peak change in mean arterial pressure =

-0.5± 2.5 versus 23.6±3.6 mm Hg, $P<0.0001$; Figure VIII.2).

All samples tested raised vascular smooth muscle calcium uptake approximately 50% from basal values, but the levels were higher in hypertensive plasma-treated rat tail arteries than in normotensive plasma-treated ones (15.5±0.8 versus 13.2±0.6 nmol/g per minute, $P<0.05$). Furthermore, in hypertensive subjects ($r=0.883$, $P<0.0002$), but not in normotensive subjects ($r=0.343$, $P<.21$), there was a strong correlation between the ability of plasma to stimulate ^{45}Ca uptake, and its ability to raise arterial blood pressure (Figure VIII.3).

D. DISCUSSION

Parabiotic experiments conducted by Dahl more than two decades ago established the humoral transmission of elevated blood pressure from a primary genetic model, the salt-sensitive S rat, to its normotensive control, the R rat (1). Since that time, circulating factors from genetic hypertensive rat models, capable of raising blood pressure and of increasing tissue calcium uptake, have been repeatedly identified by several groups (2-6). Although fewer studies have been carried out in human hypertension, there is evidence of a circulating factor, independent of circulating digitalis-like factors, that alters cellular calcium handling in platelets and neutrophils (7,8). However, the ability of these factors to elevate blood pressure has not been demonstrated.

Our group has recently identified a circulating factor, with a molecular weight greater than 1,000, present in SHR but not WKY rats, that (1) causes a delayed

(>30 minute onset) elevation in blood pressure when injected into SD rats; (2) constricts and increases ^{45}Ca uptake in the rat tail artery; (3) does not inhibit Na^+-K^+ ATPase activity; (4) appears to be parathyroid-dependent, but is not itself the parathyroid hormone; and (5) is associated with dramatic increases in a histologically novel cell type, identified in the parathyroid gland (10,11). As increased parathyroid activity has been reported in at least some patients with essential hypertension (15), it seemed reasonable to investigate the possible presence of a similar factor in clinical hypertension.

This preliminary report suggests that a similar hypertensive factor may, indeed, be present in human hypertensive subjects. The ability of hypertensive, but not normotensive, human plasma extracts to raise blood pressure suggests that the phenomenon is specific for hypertension and is not an artifact of the bioassay rat responding to dialyzed plasma of a different species. Furthermore, the action of other, more well-known, vasopressor systems cannot explain our results, since: (1) the molecular weight cut-off for the dialyzed plasma was 1,000, ruling out peptides such as vasopressin, catecholamines, angiotensin II, and low molecular weight digitalis-like factors; (2) the delayed pattern of the blood pressure response, beginning at 30 minutes and peaking after 45 to 60 minutes, is also not observed with other previously defined pressor agents; and (3) no differences in the level of plasma renin activity or average urinary sodium excretion were observed in the hypertensive compared with the normotensive subjects. Lastly, while a significant fraction of the basal ^{45}Ca uptake may not have been specifically identified, the pressor effects of the

hypertensive extracts were correlated closely with the stimulation of ^{45}Ca uptake in vascular smooth muscle (Figure VIII.3).

Although the plasma from the hypertensives taken as a group had considerable pressor activity in the rat, this was not equally true of all the hypertensive subjects studied (Figure VIII.2). Thus, consistent with the heterogeneity of human hypertension, the circulating factor identified here may not be present or relevant to all clinical forms of hypertension. Current research is focusing on the question of whether this circulating factor is unique to or predominates in different pathophysiological types of hypertensive disease (e.g. different renin subgroups of hypertension, salt-sensitive versus salt-insensitive subjects, etc.) and on the mechanism of its hypertensive action, whether involving direct end-organ effects or mediated by the production of other humoral or cellular factors.

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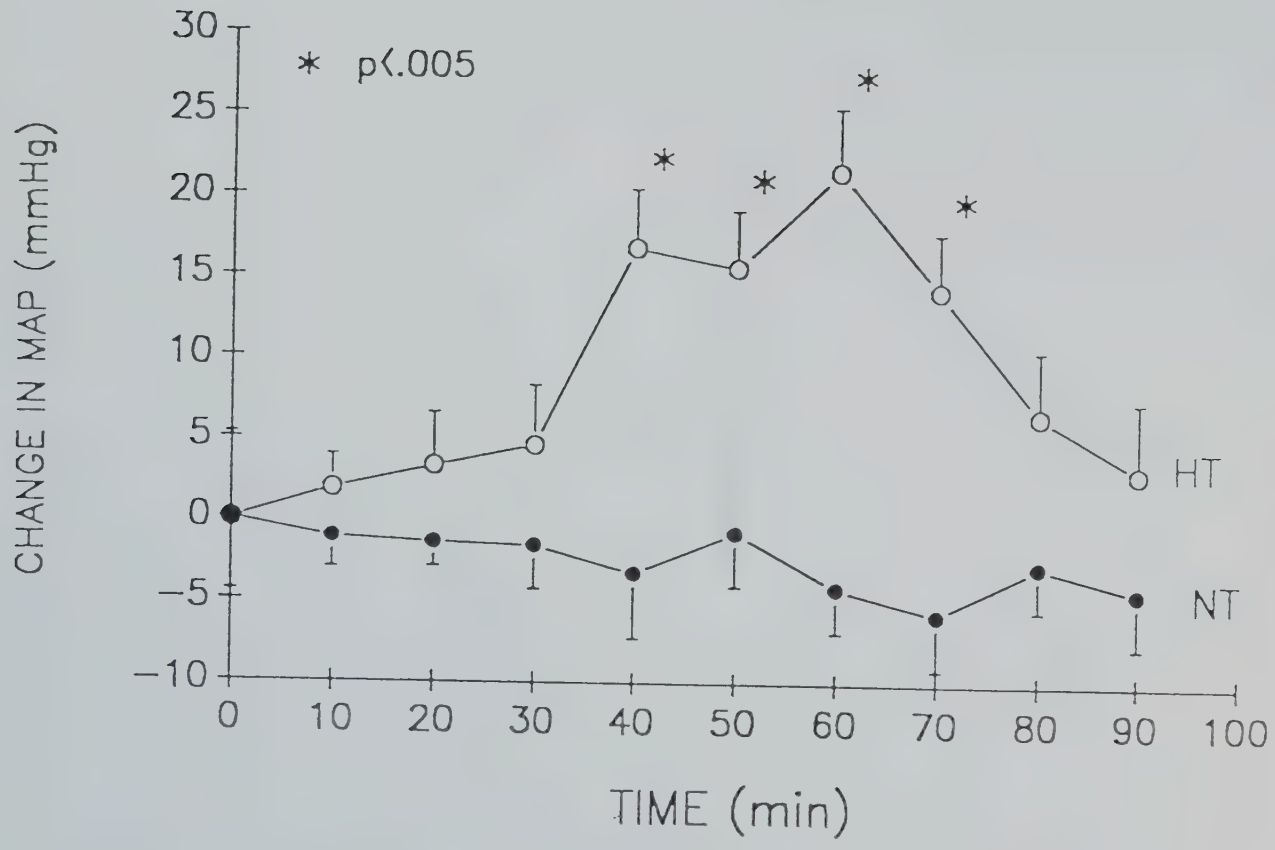

Figure VIII.1.

Time course of blood pressure responses to normotensive (NT, •) and hypertensive (HT, o) dialyzed plasma samples in the bioassay rat model. MAP, mean arterial pressure. *$P < 0.005$ versus T=0.

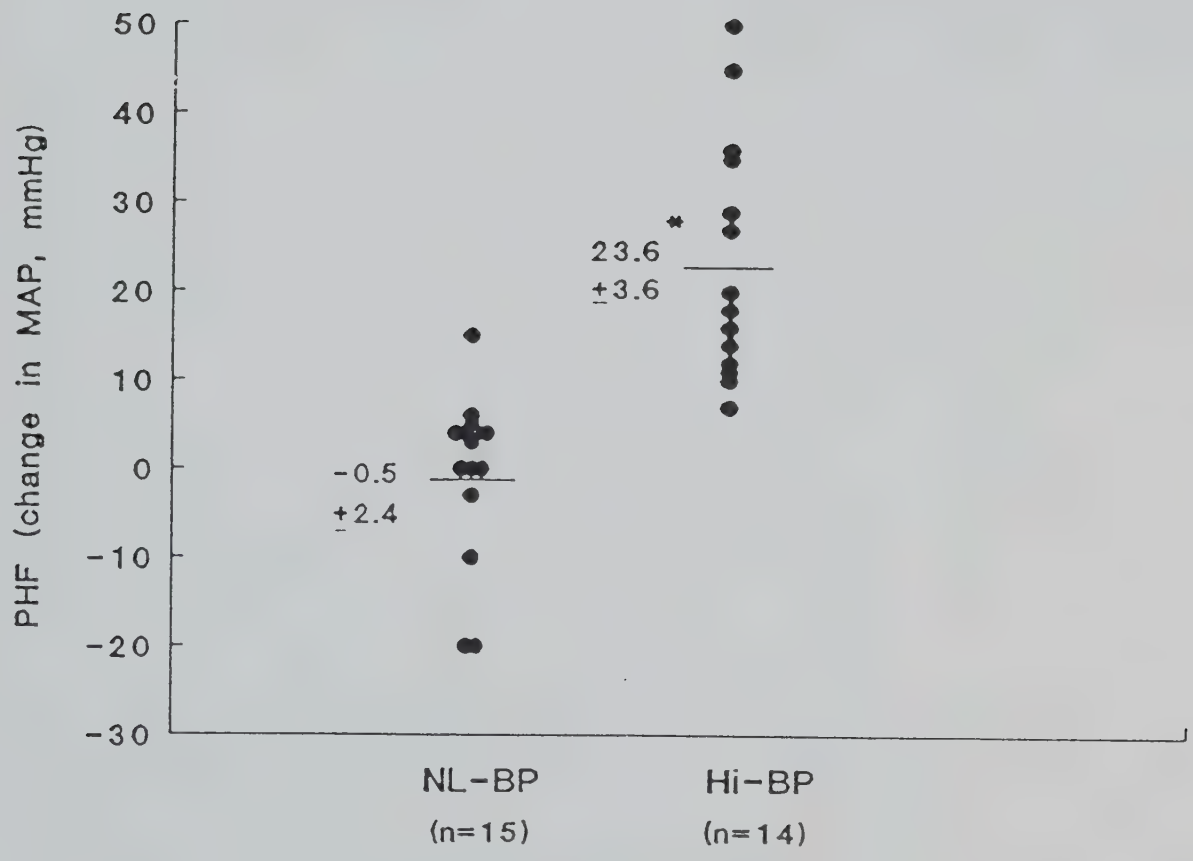

Figure VIII.2.

Parathyroid hypertensive factor levels (peak ΔMAP, mm Hg, induced by dialyzed plasma extracts) in normotensive (NL-BP) and essential hypertensive (HiBP) subjects. MAP, mean arterial pressure. $^{*}P < 0.0001$ versus normotensive subjects.

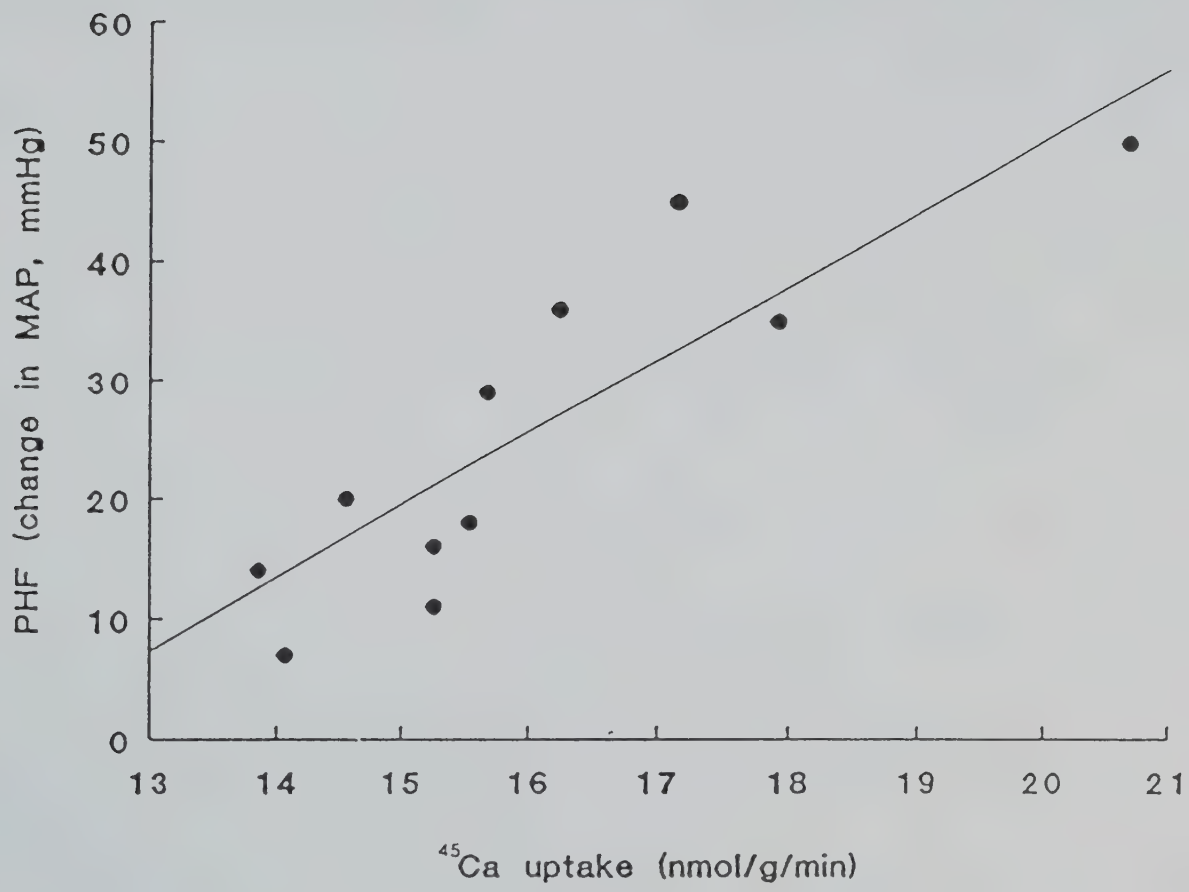

Figure VIII.3.

Relationship between parathyroid hypertensive factor levels (ΔMAP, mm Hg) and vascular smooth muscle ^{45}Ca uptake induced by hypertensive human plasma extracts. r=0.883, $P<0.0002$. MAP, mean arterial pressure.

Table VIII.1.

Clinical and laboratory data in normotensive (n=15) and hypertensive (n=14) subjects

	Age years	Race W/B	Sex M/F	SBP mm Hg	DBP mm Hg	$U_{Na}V$ mmol/l	PRA ng/ml/h	PHF ΔMAP, mm Hg
Normotensive	45±4	11/4	8/7	114±4	71±3	138±33	2.7±1.0	-0.5±2.5
Hypertensive	50±4	9/5	8/6	155*±5	100*±3	132±37	2.7±1.2	23.6*±3.6

W, white; B, black; M, male; F, female; SBP, systolic blood pressure; DBP, diastolic blood pressure; $U_{Na}V$, urinary sodium excretion; PRA, plasma renin activity; PHF, parathyroid hypertensive factor; MAP, mean arterial pressure (ΔMAP=PHF). *$P < 0.0001$ versus normotensives.

IX. PARATHYROID HYPERTENSIVE FACTOR IN HYPERTENSIVE PRIMARY HYPERPARATHYROID PATIENTS

A. INTRODUCTION

Hypertension is commonly associated with primary hyperparathyroidism, with a reported incidence of 18 to 78% in hyperparathyroid patients (1-7). The mechanism of this hypertension, however, has not been well established. The fact that not all primary hyperparathyroid patients are hypertensive makes it difficult to link causally the hypertension with elevated parathyroid hormone (PTH) or calcium levels, as has been suggested in the past. In fact, parathyroid hormone has long been known to have hypotensive properties (8-10). Post-operatively, some hypertensive hyperparathyroid patients show an improvement in blood pressure, whereas others show either no change or even a worsening of their hypertension (1-7). Thus, due to the variable effects of parathyroidectomy on hypertension in primary hyperparathyroid patients, and due to the lack of understanding of the relationship between hypertension and primary hyperparathyroidism, a recent authoritative review (11) could not recommend hypertension as an indication for parathyroidectomy in primary hyperparathyroid patients.

A version of this Chapter has been submitted for publication to Journal of Clinical Investigation

Recently, we have described the presence of a novel circulating hypertensive factor in the plasma of Spontaneously Hypertensive Rats (SHR) (12). This factor increases calcium uptake in vascular smooth muscle (12) and potentiates the actions of certain pressor substances such as norepinephrine, angiotensin II and vasopressin (13). In these SHR rats, parathyroidectomy has been shown to lower blood pressure and result in the disappearance of the hypertensive factor from their plasma (14). Similarly, in a series of cross-transplantation experiments, hypertension developed in parathyroidectomized normotensive rats receiving SHR parathyroid glands, but was attenuated in parathyroidectomized SHR rats receiving normotensive rat parathyroid glands. This phenomenon has recently been confirmed in stroke-prone SHR rats (15). In the former study, the hypertensive factor became detectable in the plasma of the former group and disappeared from the plasma of the latter group (14). Finally, when parathyroid glands from SHR were grown in primary culture, media from the culture was found to contain typical hypertensive factor activity (16). On this basis, we have termed the factor Parathyroid Hypertensive Factor, or "PHF".

PHF activity has since been found in a proportion of patients with essential hypertension (17). Based on the parathyroid origin of the factor in SHR, we hypothesized that PHF might thus be responsible for a proportion of the hypertension in primary hyperparathyroidism. In this report, we studied primary hyperparathyroid patients for the presence of PHF and examined the effect of parathyroidectomy on such patients.

B. METHODS

Patients

This study was carried out in patients referred to the University of Alberta Hospital with a tentative diagnosis of primary hyperparathyroidism. Patients reported are those with surgically proven parathyroid adenomas. Blood pressures reported represent the means of values obtained on at least two different occasions, both pre- and post-operatively. For consistency, in any single patient, blood pressure was measured by the same observer - either one of us (RZL) or the patient's regular physician. In some cases where the values used were those recorded by the patient's family physician, the approximate level of blood pressure was confirmed by us, both pre- and post-operatively. Where possible, blood pressures were measured off all antihypertensive medications. In one case this was not possible, and therefore blood pressure post-operatively was measured on the same dose of antihypertensive medication as preoperatively. Post-operative blood pressure values used were those obtained 1 to 6 months after surgery. Hypertension was defined, according to JNC III criteria, as a diastolic pressure of >90 or a systolic pressure of >160 (18). In cases where measurement of pre- and post-operative blood pressure was not done under standardized conditions (i.e. done by different observers or antihypertensives not controlled for), patients were excluded from assessment of post-operative change in blood pressure, although they were used in the assessment of preoperative PHF levels and blood pressure. On this basis, five hypertensive patients were studied off antihypertensive medications, one was studied on the same dose of antihypertensive

medication pre- and post-operatively, and four were excluded from blood pressure analysis because reported changes in blood pressure could not be verified or were not measured on more than one occasion. Four normotensive patients were also excluded from blood pressure analysis for the latter reasons.

Biochemical Assays

Total calcium was measured by automated methods and represents the mean of at least two separate determinations. Such measurements were corrected for albumin binding according to the formula: total calcium = measured calcium + [(40-albumin) × .02], where calcium was measured in mM and albumin was measured in mg/dl. Intact PTH was measured using a commercial kit (Nichols Institute, San Juan Capistrano, CA).

PHF Determinations

PHF levels were measured by bioassay, as previously described (12). Briefly, male Sprague-Dawley rats, weighing 300 to 350 g, were anesthetized (pentobarbital, 50 mg/kg intraperitoneally) and underwent cannulation of a carotid artery and jugular vein. After a stable blood pressure was established, test plasma at a dose of 4.5 ml/kg was injected intravenously. Mean arterial pressure (MAP) was monitored for 90 minutes thereafter. PHF was quantitated (in mm Hg) as the peak of the characteristic delayed, prolonged increase in blood pressure seen 30 to 75 minutes post-injection. Such an increase would normally begin 30 min post-injection, peak at

around 50 minutes and return to baseline by 90 minutes. In cases where blood pressure decreased, the highest sustained MAP between 30 and 75 minutes was used to calculate the PHF value, even though a negative number might be obtained. All assays were performed in duplicate, with one rat being used per assay. Assays were interpreted by two observers blinded to the patient's blood pressure status.

Statistics

Changes in blood pressure in individual patients and the results of the bio-assays in individual rats were assessed using a two-tailed paired *t*-test. Differences between hypertensive and normotensive groups were assessed using two-tailed unpaired *t*-tests. Effects of various variables in predicting post-operative changes in MAP, as well as correlations between various variables, were calculated by multiple linear regression using GB-STAT (Dynamic Microsystems, Inc., Silver Spring, MD). Results are reported as the mean$\pm$SEM. A P of $<.05$ was considered significant.

C. RESULTS

Patient characteristics are shown in Table IX.1. Diastolic, systolic and mean arterial pressures were all higher in the hypertensive compared to the normotensive group. Age, total calcium and intact PTH levels were not different between the two groups.

Figure IX.1 shows individual PHF values in the normotensive and hypertensive groups. There was virtually no overlap in PHF values in the two groups, with

the exception of one hypertensive patient whose plasma consistently induced a decrease in blood pressure in our bioassay system. Accordingly, this patient's PHF level was assigned a "negative" value, as described in the Methods section. In general, the mean normotensive PHF level was close to zero (0.6 ± 1.8 mm Hg, $P = .64$), whereas the mean PHF level in hypertensives was 11.6 ± 2.4 mm Hg ($P < .0001$). Thus, based on this small sample, most hypertensive primary hyperparathyroid patients would seem to have significant plasma PHF levels. In contrast, no normotensive patients had significant plasma PHF levels (arbitrarily considered as > 5 mm Hg by us, based on the variability of the bioassay).

Reliable data for post-operative changes in blood pressure were available in twelve cases. In Figure IX.2, the effect of parathyroidectomy on MAP is shown. As can be seen, parathyroidectomy had no consistent effect in PHF "negative" patients, whereas PHF "positive" patients consistently showed a decrease in MAP. This decrease in MAP reflected both a fall in mean systolic and diastolic pressures of 25 ± 8 ($P < .01$) and 12 ± 4 mm Hg ($P < .01$), respectively. It should be pointed out, however, that two of the six hypertensive PHF-positive patients still remained, by definition, hypertensive, despite a fall in their blood pressures. On the other hand, the single hypertensive patient who was PHF-negative showed no decrease in MAP post-operatively. In fact, the immediate post-operative course in this patient was complicated by severe hypertension, requiring urgent antihypertensive therapy.

In order to determine which factors might predict post-operative changes in blood pressure, PHF level, preoperative MAP, total calcium and PTH levels were

entered as independent variables into a multiple linear regression analysis. Of these factors, only the PHF levels predicted the change in MAP post-operatively. This relationship is plotted in Figure IX.3. Using single linear regression, preoperative MAP correlated inversely (correlation coefficient = -.680, $P < .05$) with post-operative change in MAP. However, preoperative MAP was also significantly related to the PHF level (correlation coefficient = .732, $P < .01$); thus, its effect was minimized in the multiple linear regression.

Post-operative PHF levels were available in six out of nine PHF-positive hypertensive patients. The mean preoperative PHF level was 10.8, with a range of 5 to 25. The mean post-operative value was -4.6, with a range of 0 to -25. Thus, PHF levels were seen to normalize in all PHF-positive hypertensive patients post-operatively (Figure IX.4).

D. DISCUSSION

The results of this study show that a significant proportion of hypertensive primary hyperparathyroid patients possess a circulating hypertensive factor -- PHF. In such patients, PHF seems to be causally linked to the hypertension, as suggested by the association between MAP and PHF, and by the correlation between the pre- and post-operative changes in MAP and the preoperative PHF value. Although the correlation between MAP and PHF could potentially represent an epiphenomenon, our previous parathyroid cross-transplantation studies suggest that this is not the case (14). Moreover, in controlled calcium feeding experiments in SHR (19) and in

DOCA-salt rats (20), an increase in PHF could be seen to precede an increase in MAP. Certainly, PHF is not the cause of all hypertension in primary hyperparathyroidism, as demonstrated by the one hypertensive "PHF-negative" patient in the current series. One might expect that patients with non-PHF related hypertension may also develop hyperparathyroidism, and these, of course, would not necessarily be expected to show PHF activity. It is, in fact, surprising that there were not more PHF-negative hypertensive patients. Based on previous reports, slightly less than half of hypertensive primary hyperparathyroid patients show a decrease in blood pressure post-operatively, although the reported response rate varies from 0 to 74% (1,2,4-7). Why such a high proportion of patients in our series showed a decrease in blood pressure is subject to speculation. In two patients, systolic blood pressure decreased without a substantial change in diastolic blood pressure; in two other patients, the converse was true. In addition, two hypertensive patients remained, by definition, hypertensive post-operatively, despite an appreciable drop in MAP. Thus, varying criteria for a significant fall in blood pressure in previous series may have influenced reported outcomes. In fact, most previous studies do not define what was considered to be a significant change in blood pressure. In other cases, the individual changes are averaged, possibly obscuring the hypotensive effect of parathyroidectomy in some patients by the hypertensive effect in others. Finally, the time of follow-up has varied in previous reports, making it difficult to compare the various studies. Nevertheless, in the current study, PHF does seem to be involved in most cases of hypertension in primary hyperparathyroidism.

The results of this study show no relationship between PTH and MAP, nor does PTH predict post-operative changes in blood pressure. Thus, PTH would not seem to be responsible for the hypertension of primary hyperparathyroidism. Furthermore, PHF probably does not represent a fragment of PTH, as N-terminal-deleted (14) and other PTH fragments (21) have had no hypertensive effect in our hands. Similarly, PHF is apparently unique from other substances produced by the parathyroid gland, such as chromogranin A or the humoral hypercalcemic factor, as both of the latter are also hypotensive (22,23).

The ability of PHF to be predictive of the post-operative blood pressure change in primary hyperparathyroid patients may prove to be clinically useful in light of the previous discussion. It may allow a prediction to be made as to the potential effect of parathyroidectomy on blood pressure in asymptomatic primary hyperparathyroid patients. In this series, preoperative MAP could also statistically predict post-operative change in MAP, but this effect is likely due to the high number of PHF-positive patients. Thus, from a predictive point of view, the PHF level is a more accurate predictor of post-operative blood pressure change. This is because the effect of parathyroidectomy on hypertensive PHF-negative patients would be accurately predicted by the PHF levels, but not by the preoperative MAP. In a population with a greater proportion of hypertensive PHF-negative patients, preoperative MAP would therefore lose its predictive value.

This study also shows that PHF expression is associated with a state of parathyroid hyperfunction in humans. It is further known that a relative state of secondary

hyperparathyroidism exists in low-renin essential hypertension (24) and that PHF expression is also associated with this condition (25). As well, SHR rats, who produce PHF, have been shown to have parathyroid hyperplasia (26). What the stimulus is for PHF expression in such circumstances is unknown and will require further study. It is therefore interesting to note that essential hypertensive patients also have an increased probability of developing primary hyperparathyroidism (27). Whether this represents the development of autonomy in hyperplastic parathyroid tissue is unknown. Presumably, in most primary hyperparathyroid patients, the hypertension occurs as a consequence of the hyperparathyroidism, otherwise a significant number of asymptomatic primary hyperparathyroid patients would present with hypertension rather than hypercalcemia.

In summary, the parathyroid glands in SHR rats produces a hypertensive substance -- PHF. PHF is also found in human essential hypertension and coexists with enhanced parathyroid activity. Results of this study show that PHF may be responsible for a proportion of the hypertension seen in primary hyperparathyroidism. Additionally, based on the disappearance of PHF post-operatively in PHF-positive hypertensive patients, evidence is obtained for the parathyroid origin of PHF in some humans. Currently, work is underway on human parathyroid tissue to confirm this as a source of PHF production and to study controlling factors in PHF expression.

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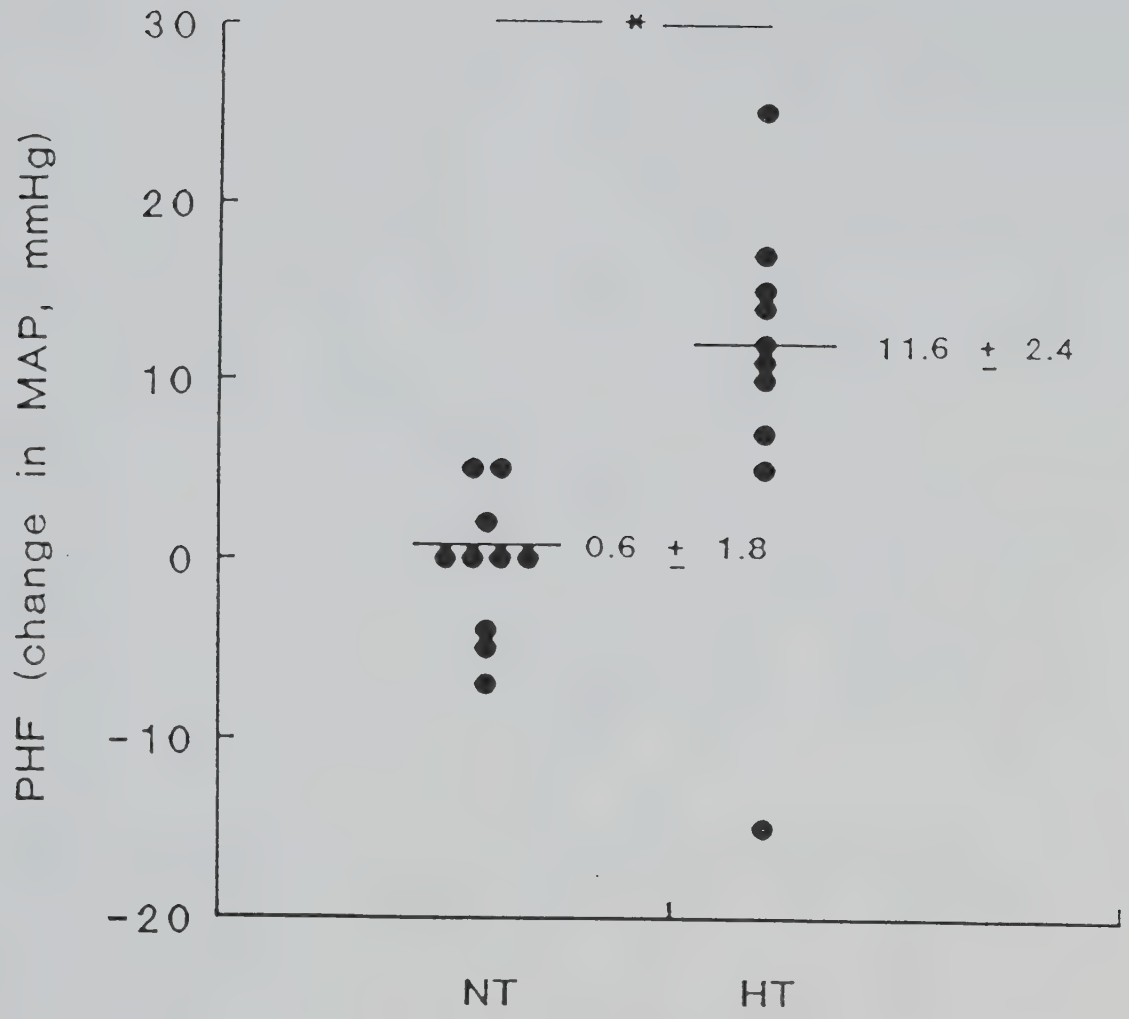

Figure IX.1.

PHF levels in individual patients. Note one hypertensive patient with a "negative" PHF value. * denotes $P = .003$ by two-tailed t-test for the comparison between groups. NT = normotensive, HT-hypertensive.

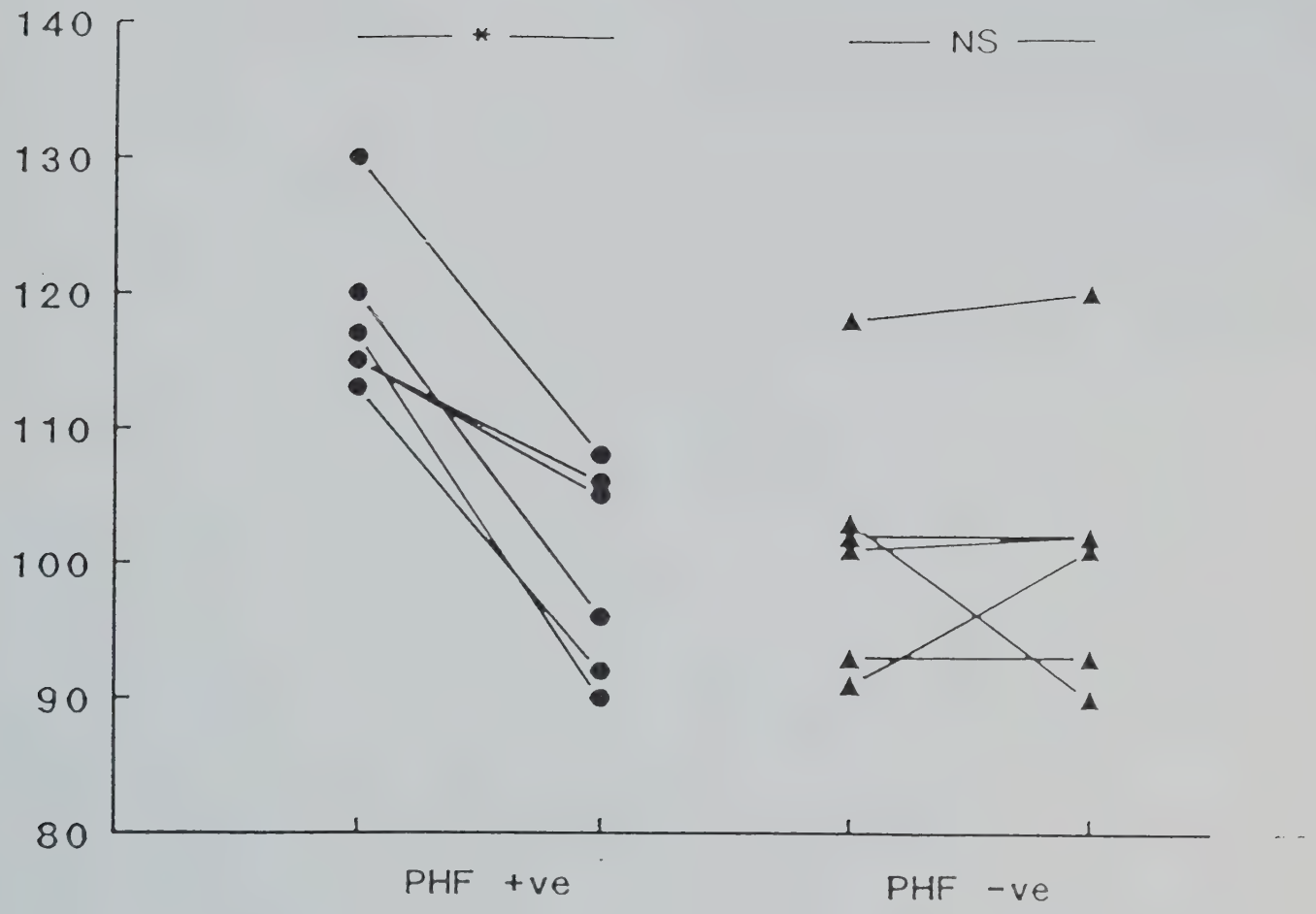

Figure IX.2.

Changes in mean arterial pressure in PHF-positive and PHF-negative primary hyperparathyroid patients after parathyroidectomy. * denotes $P = .0017$ by two-tailed paired t-test for post-operative change in individual patients.

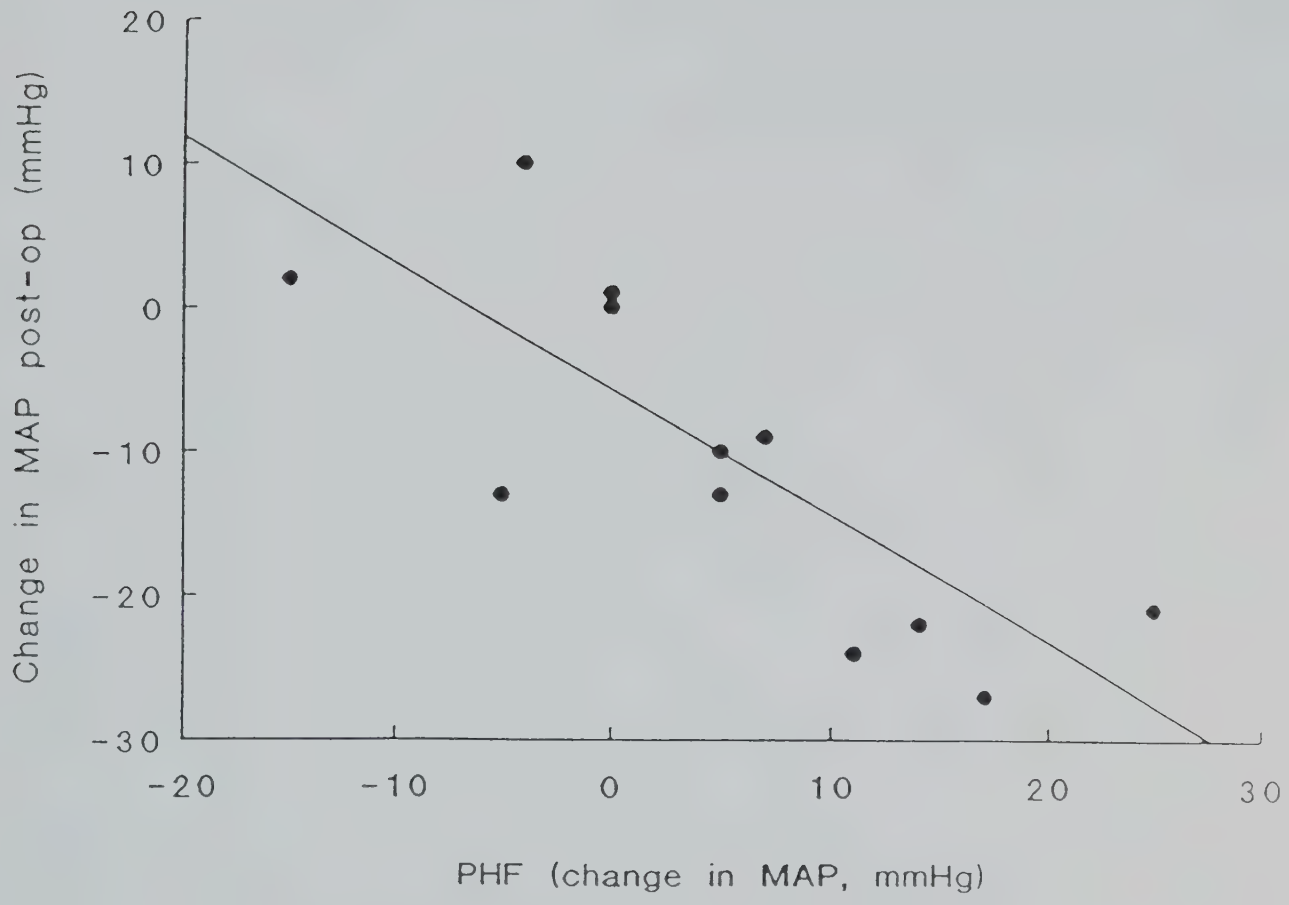

Figure IX.3.

Relationship between preoperative PHF level and post-operative change in blood pressure. r=-.789, *P*=.0016

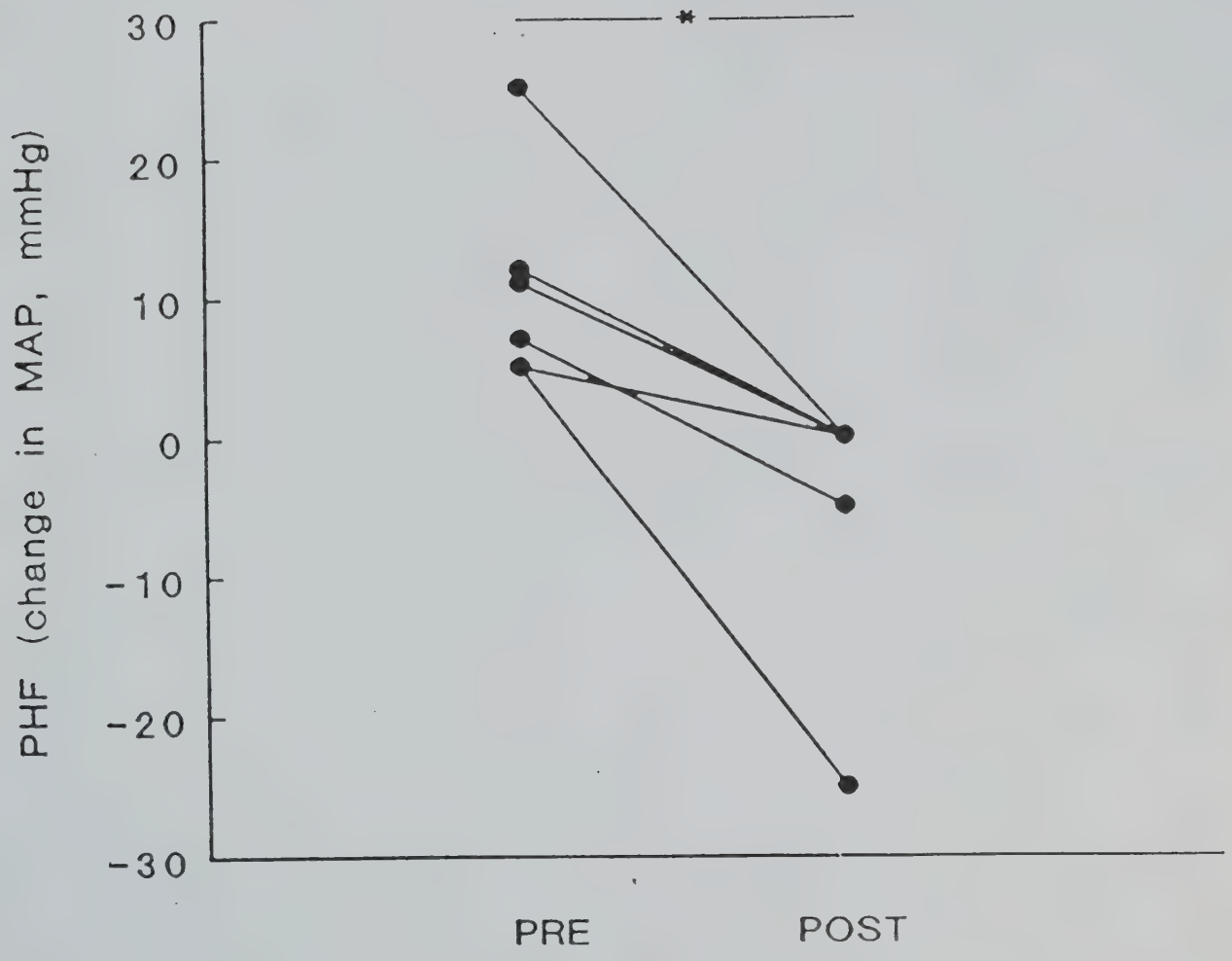

Figure IX.4.

PHF levels pre- and posto-peratively. * denotes $P = .0097$ by two-tailed paired t-test for post-operative change in individual patients.

Table IX.1.

Preoperative characteristics of hypertensive and normotensive primary hyperparathyroid patients.

Characteristic	Hypertensive (n=10)	Normotensive (n=10)	*P* Value
Male	2	2	NS
Female	8	8	NS
Age (yr)	49	54	NS
Mean arterial pressure (mm Hg)	116.0±2.9	96.3±2.4	<.0001
Diastolic blood pressure (mm Hg)	95.3±1.6	78.9±2.1	<.0001
Systolic blood pressure (mm Hg)	157.6±7.9	130.9±3.8	.016
PHF (Δ MAP, mm Hg)	11.6±2.4	0.6±1.8	.003
PTH (pg/ml)	86±7	150±40	NS
Ca^{++} (mM)	2.70±.07	2.70±.02	NS

P values for comparisons between groups. NS = not significant

X. PARATHYROID HYPERTENSIVE FACTOR IS PRESENT IN DOCA-SALT BUT NOT TWO KIDNEY-ONE CLIP HYPERTENSIVE RATS

A. INTRODUCTION

Parathyroid Hypertensive Factor (PHF) is a newly described circulating hypertensive factor found in Spontaneously Hypertensive Rats (1) and in a proportion of human hypertensive patients (2). In SHR, interventions which alter blood pressure, such as parathyroidectomy (3) or alterations in dietary calcium (4), are associated with a change in PHF levels in the direction of change of the blood pressure. Although such observations can be used as evidence for a causal role of PHF in some forms of hypertension, the possibility exists that changes in PHF levels are secondary to the changes in blood pressure. As PHF appears to be of parathyroid origin in SHR (3) and is affected by calcium intake, and as low renin hypertension is also characterized by alterations in parathyroid function (5) and by sensitivity to dietary calcium (6), we hypothesized that if PHF played a causative role in hypertension, it should be present mainly in low renin forms of hypertension. If, on the other hand, PHF expression is secondary to hypertension, it should be present in

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both low and high renin forms of hypertension. The purpose of this study, therefore,was to determine PHF expression in low renin (DOCA-salt) and high renin (two kidney-one clip) models of hypertension, using the same rat strain in both cases.

B. METHODS

DOCA-Salt Hypertensive Rats

Male Wistar-Kyoto (WKY) rats (Charles River, Wilmington, MA) aged 12 weeks were used in this study. Rats were anesthetized and underwent unilateral nephrectomy with subcutaneous implantation of a deoxycorticosterone acetate (DOCA) -impregnated silastic rubber patch, trimmed to contain 50 mg/kg of DOCA per rat (n=15). Following recovery from surgery, rats were given a 1% NaCl solution to drink and were allowed free access to standard rat chow. Controls for this experiment included a uninephrectomized-saline group implanted with a silastic patch not containing DOCA (n=9), and a uninephrectomized-DOCA patch-distilled water group (n=8).

Two Kidney-One Clip (2K-1C) Hypertensive Rats

Male WKY rats aged 12 weeks, from the same cohort as above, were used. Rats were anesthetized and underwent placement of a silver kidney clip around the left renal pedicle (n=14). The pedicle had previously been cleared of perinephric fat by blunt dissection. Controls consisted of rats which had the renal pedicle exposed but in which no kidney clip had been placed (n=8). Rats were allowed free access

to tap water and standard rat chow during the experimental period.

Blood Pressure Measurement

Mean arterial pressure (MAP) was measured in a random group of rats at the beginning of the study (control, n=12), in the DOCA groups after 4 weeks, and in the two kidney-one clip group after 6 weeks. These latter times were chosen to allow for full expression of the hypertension. At these times, silastic implant DOCA-salt rats are known to have achieved maximum blood pressure and are in a low renin state (7). Slightly longer was allowed for 2K-1C rats in order to ensure that maximum blood pressure would be achieved in this model. Nevertheless, 2K-1C rats are known to be in a high renin state at 6 weeks post-clipping (8). MAP was measured in the various groups in the following manner. Rats were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and underwent cannulation of a carotid artery. MAP was recorded until a stable level of at least 5 minutes duration was achieved. Five ml of blood were then withdrawn into heparinized syringes (100 IU heparin/ml blood) and the rats sacrificed by pentobarbital overdose. The collected blood was immediately chilled (4°C), centrifuged and the plasma dialyzed for 24 hours against saline at 4°C using 1,000 molecular weight cut-off dialysis tubing. The resultant plasma was either assayed for PHF immediately or stored at -20°C and assayed within 2 weeks.

PHF Assay

PHF was assayed as previously described (1). Briefly, male Sprague-Dawley rats weighing 300 to 350 g were anesthetized with pentobarbital (50 mg/kg intraperitoneally) and underwent cannulation of a carotid artery and jugular vein. MAP was continuously monitored via the carotid artery catheter. Previously obtained dialyzed plasma samples were injected intravenously at a dose of 4.5 ml/kg. PHF activity was quantitated as the peak of the characteristic delayed, prolonged increase in blood pressure occurring approximately 30 to 70 minutes after injection, with the peak being at about 50 minutes post-injection. In cases where no PHF-type increase in MAP was detected, the MAP recorded was the highest sustained blood pressure obtained between 40 to 60 minutes after plasma injection.

Statistics

Comparisons of MAP between groups was carried out by one-way analysis of variance using the Student-Newman-Keuls test for post-hoc comparisons. The presence or absence of PHF was assessed by paired t-test based on the null hypothesis that change in MAP following plasma injection should equal zero. Results are expressed as the mean$\pm$SEM.

C. RESULTS

MAP in the control group of rats at the beginning of the experiment was 92$\pm$7 mm Hg. Figure X.1 shows MAPs of the control group of rats at the beginning

of the experiment and rats in the various experimental groups at the end of the experiment. From this figure, it is apparent that blood pressure was significantly elevated in both the DOCA-salt and 2K-1C rats. No statistically significant increases were seen in nephrectomy-saline, DOCA-distilled water or sham groups. As seen in Figure X.2, DOCA-salt, but not 2K-1C, rats showed significant levels of PHF, despite nearly identical MAPs. Interestingly, DOCA-distilled water rats also had significant PHF levels, even though MAP was not significantly increased. No other groups of rats had significant PHF levels. In the DOCA-salt rats, MAP correlated significantly with PHF levels ($r = .91$, $P < 0.001$), as shown in Figure X.3. No other group showed a significant correlation between the two variables.

D. DISCUSSION

Both the interventions of kidney clipping and DOCA-salt increased blood pressure by an equivalent amount. These increases were specific to the interventions, as no other groups of rats showed a significant increase in blood pressure over levels in the initial control group. From the mean levels and standard errors, however, it can be seen that the increase in blood pressure in the DOCA-saline rats was not as uniform as expected. We are not sure as to the reason for this. Possibly there was variable absorption of the DOCA between rats. Alternatively, some rats may not have reached peak MAP by 4 weeks, as this is the generally accepted time that a plateau of peak MAP is reached in DOCA-salt rats. Nevertheless, the variability in blood pressure is somewhat fortuitous. Specifically, it allowed a correlation to be

observed between MAP and PHF levels. This would tend to rule out measurement error as the cause of variance in MAPs and suggests that PHF may be causally linked to the development of hypertension in the DOCA-salt rats. This conclusion is supported by the 2K-1C data. Despite an equivalent mean increase in blood pressure in both DOCA-saline and 2K-1C rats, only the DOCA-saline rats showed PHF activity in their plasma. Thus, it can be concluded that PHF secretion is not a non-specific response to hypertension *per se*, but seems to be linked to the low renin state. Additional support for this conclusion is provided by data from the DOCA-distilled water group. Despite no significant increase in MAP, low, but significant, levels of PHF were detected. This is presumably due to the availability of NaCl in our standard rat chow (0.4% NaCl). Ideally, it would have been useful to measure plasma renin activity in the various groups of rats to confirm the renin-PHF relationship. Unfortunately, this was not done, but is planned for a future experiment. Nevertheless, the fact that PHF is detected prior to the onset of hypertension again supports the causative role of PHF in some forms of hypertension. Indeed, in a prior study involving calcium feeding of SHR, PHF was also detected prior to the onset of hypertension (4). Finally, it can be noted that even in uninephrectomized rats, 1% NaCl in the drinking water was insufficient to increase blood pressure or induce the expression of PHF. This supports the work of others showing that a high salt diet alone does not uniformly increase blood pressure in normotensive individuals (9, 10).

In this study, it is significant to note that a normotensive rat strain, namely the WKY, has the capacity to produce PHF. This leads to the question of whether PHF

might be expressed in low amounts in normal animals. Such expression would suggest a physiological role for PHF. Given the lack of sensitivity of an *in vivo* bioassay, we are unable to say at present whether PHF is expressed in otherwise normal animals or humans. In the current experiment, uninephrectomy and saline were insufficient to induce detectable PHF expression in WKY rats. Additionally, we have maintained WKY rats on a high salt and low calcium diet for 2 months with no expression of PHF (Choi, Lewanczuk and Pang, unpublished data). Thus, perhaps this study further supports the widely held notion that a genetic predisposition to hypertension is necessary before environmental factors become significant. Given the results of this experiment, such a genetic predisposition could include abnormalities in mineralocorticoid metabolism, as has been previously suggested (11). Clearly, mineralocorticoid was necessary for PHF expression in this study; the mechanism of this effect remains to be elucidated.

Finally, results of this study correlate with human studies showing that PHF is present in low renin, but not high renin, essential hypertensive patients (12). Moreover, our previous studies have shown that PHF appears to be of parathyroid origin based partially on the observations that PHF activity disappears from the plasma after parathyroidectomy in SHR (3). It is, therefore, significant to note that it has also been reported that parathyroidectomy in DOCA-salt rats also attenuates the degree of hypertension (13). Furthermore, DOCA-salt rats display the calcium hormone-related abnormalities characteristic of low renin essential hypertensive patients and SHR rats (14). Finally, dietary calcium has been reported to decrease

blood pressure in DOCA-salt rats (14, 15), as has been reported for SHR (16), and which we have shown suppresses PHF secretion (4).

In conclusion, PHF appears to be specific for low renin, but not high renin, hypertension. In DOCA-salt rats, PHF levels correlate with MAP and PHF appearance seems to antedate the onset of hypertension. Thus, PHF may be causally linked to low renin forms of hypertension. This certainly does not imply that PHF is the sole cause of hypertension in such animals or patients; rather, PHF may variably contribute to hypertension in various circumstances. Clearly, further work will be needed to determine the significance of PHF in the various forms of hypertension.

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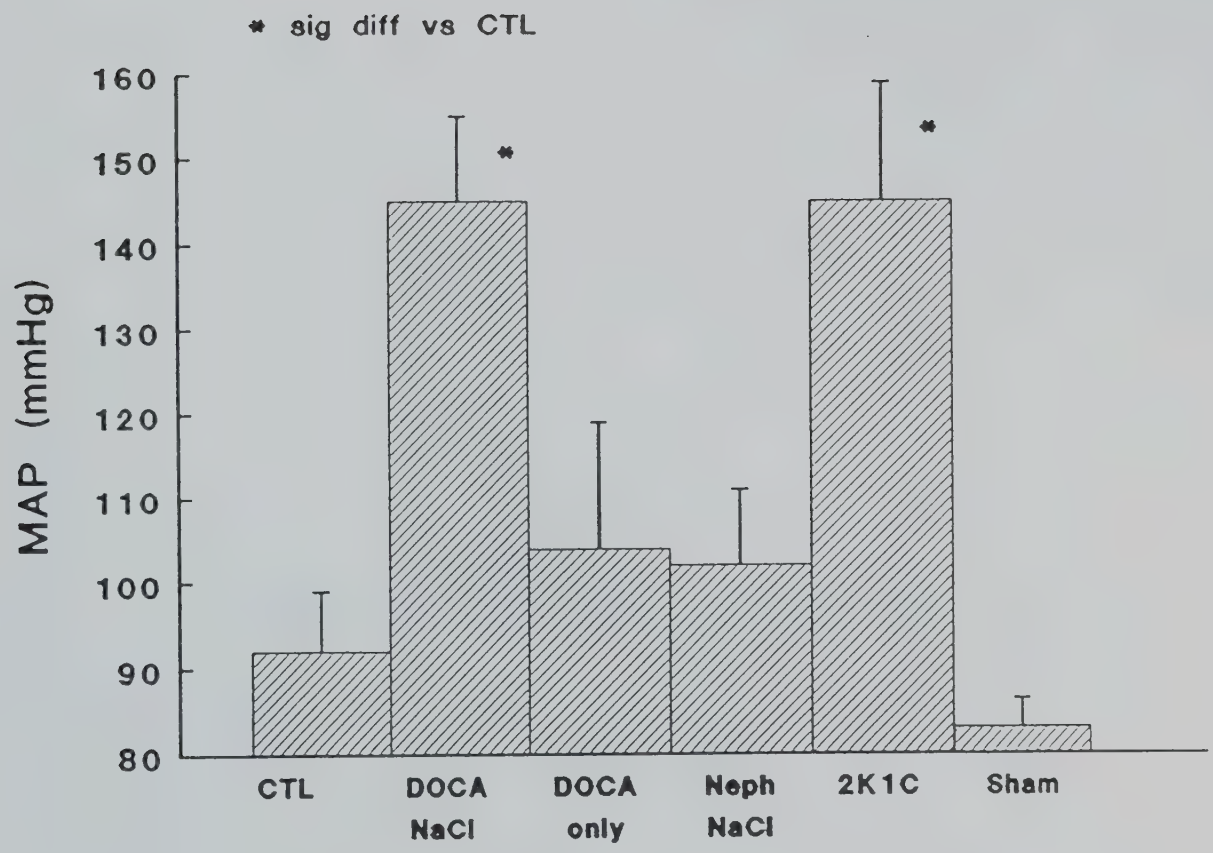

Figure X.1.

Mean arterial pressures in experimental groups. CTL=control; Neph-NaCl= uninephrectomized, saline-drinking. $*P<.01$ vs CTL, $**P<.05$ vs 0, $+P<.0001$ vs 0.

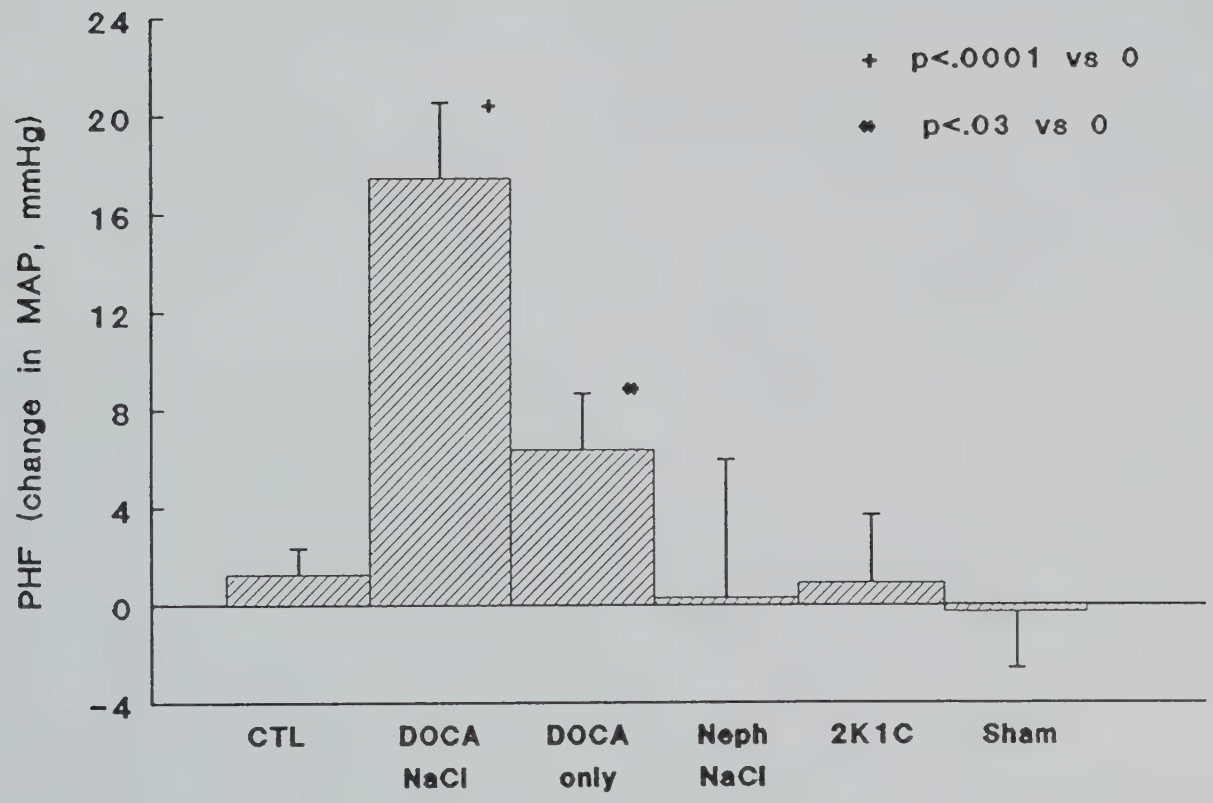

Figure X.2.

PHF levels in experimental groups. CTL=control; Neph-NaCl=uninephrectomized, saline-drinking. $*P<.01$ vs CTL, $**P<.05$ vs 0, $+P<.0001$ vs 0.

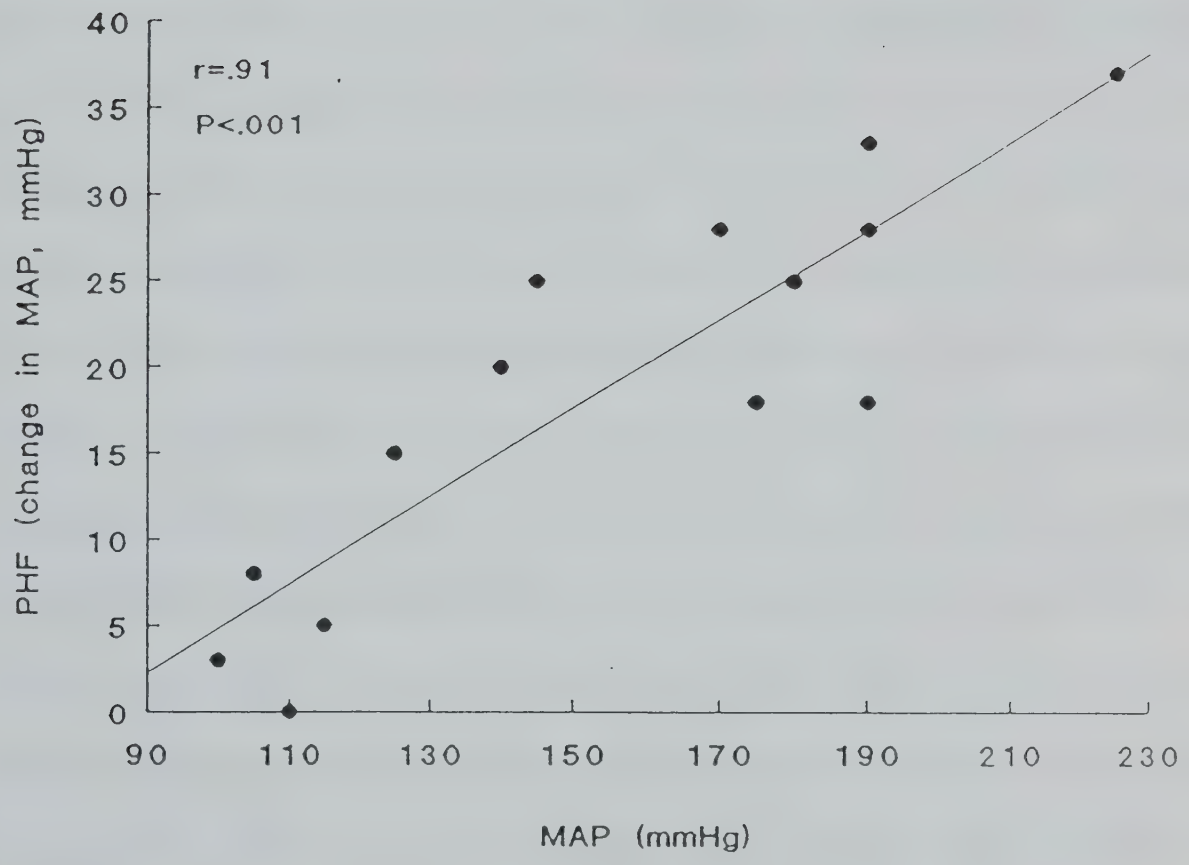

Figure X.3.

Relationship between PHF level and MAP in DOCA-salt rats.

XI. THE OCCURRENCE OF PARATHYROID HYPERTENSIVE FACTOR IN DAHL RATS

A. INTRODUCTION

Parathyroid Hypertensive Factor (PHF) is a circulating hypertensive factor of parathyroid origin (1,2). It was first described in the plasma of Spontaneously Hypertensive Rats (SHR) and has since been found in the plasma of DOCA-salt rats (3) and low renin, salt-sensitive essential hypertensive patients (4,5). Dahl-S rats are also thought to manifest a circulating hypertensive factor. Such an hypothesis is based on Dahl's original parabiosis experiments wherein Dahl-R rats linked in parabiosis with Dahl-S rats developed hypertension when both animals were maintained on high salt diets (6). Subsequently, further experiments have shown that administration of serum from hypertensive Dahl-S rats could increase blood pressure in normotensive Dahl-R rats (7) and that plasma from Dahl-S rats could increase calcium uptake in vascular smooth muscle (8). This putative humoral hypertensive factor in Dahl-S rats has not yet been identified, and attention has largely shifted to the role of the kidney and the autonomic nervous system in the pathogenesis of hypertension in Dahl-S rats. Because Dahl-S rats are known to have a low renin form of hypertension (9) and are,

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A version of this Chapter has been submitted for publication to Hypertension

by definition, salt-sensitive, and because PHF is associated with these forms of hypertension and has also been shown to increase calcium uptake in vascular smooth muscle (8), it was hypothesized that PHF might be associated with Dahl-S hypertension. The purpose of this study, therefore, was to determine whether Dahl-S rats expressed PHF-like activity in their plasma and to determine whether the expression of such activity could be affected by salt intake.

B. METHODS

Five week old Dahl-S and Dahl-R rats (Brookhaven strain) were obtained from Harlan Sprague-Dawley (Indianapolis, IN). Rats from each strain (S or R) were randomly divided into four groups of twelve rats each. One group from each strain underwent measurement of blood pressure and determination of PHF levels as described below. The remaining groups were placed on one of three levels of dietary sodium intake: $<0.02\%$ (low salt), 0.74% (normal salt) and 8.0% (high salt). All food was obtained from ICN Biomedicals (Costa Mesa, CA). Rats were allowed free access to the food and to tap water during the course of the study.

After 8 weeks on the respective diets, the rats were anesthetized with pentobarbital (65 mg/kg, intraperitoneally) and the mean arterial pressure (MAP) measured by means of carotid artery cannulation. Blood was then removed through the arterial catheter into syringes containing heparin, 100 IU/ml blood. Plasma was separated and dialyzed at 1,000 molecular weight cut-off against saline for 24 hours at 4°C. The resultant plasma was then stored at -20°C until assayed for PHF activity.

PHF activity was measured as previously described (1). Briefly, male Sprague-Dawley rats weighing 300 to 350 g were anesthetized (pentobarbital, 65 mg/kg intraperitoneally) and a carotid artery and jugular vein cannulated. After a stable blood pressure was ensured, plasma at a dose of 4.5 ml/kg was slowly injected intravenously. MAP was then continuously monitored for at least 90 minutes following injection. PHF was quantitated (in mm Hg) as the difference between the peak of the sustained increase in MAP -- characteristically beginning approximately 30 minutes following plasma injection, and returning to baseline by 75 minutes post-injection -- and baseline MAP. Such an increase in pressure normally peaked at 50 minutes post-injection. In cases where no increase in blood pressure was noted, PHF was quantitated using the highest sustained MAP obtained 40 to 60 minutes post-plasma injection, even though a negative number might be obtained. This bioassay has now been repeated by others (10) and a representative tracing is shown in Figure XI.1.

All results are expressed as the mean$\pm$standard error. Comparisons within strains were carried out by one-way analysis of variance using the Student-Newman-Keuls test for *post hoc* analysis. Comparisons between strains at a given time were carried out using unpaired, two-tailed *t*-tests. The presence of PHF was assessed within individual rats by paired *t*-tests based on the null hypothesis that change in MAP in the absence of PHF should equal 0. Linear and multiple regression was carried out to assess the relationship between PHF and body weight in determining MAP. All calculations were carried out using the GB-STAT computer program (Dynamic Microsystems, Inc., Silver Spring, MD). A P value of $<.05$ was considered

significant.

C. RESULTS

The weights of animals in the various experimental groups are given in Table XI.1. Prior to the start of the experiment, the weights of the Dahl-R rats were slightly greater than those of the Dahl-S rats. Nevertheless, the MAP's of the two groups were comparable (see Figure XI.2). After 8 weeks on the various diets, all rat groups showed significant increases in weight, although the weight gains were significantly attenuated in the low salt groups. Within the normal salt diet, there was no difference in body weights between Dahl-S and Dahl-R rats. On the high salt diet, however, the weight of Dahl-R rats was somewhat reduced compared to both Dahl-S on high salt and to Dahl-R on normal salt. Despite this reduction in body weight, MAP of Dahl-R on high salt was not different from Dahl-R on a normal salt diet. As body weight is known to affect blood pressure, it could not be used as a covariate. Nevertheless, within the normal and high salt groups, multiple regression analysis showed no effect of weight on MAP nor PHF. Thus, the increased MAP for Dahl-S rats versus Dahl-R rats on high salt cannot be explained by a weight difference. Furthermore, a significant difference in MAP already existed between Dahl-S and Dahl-R rats on a normal salt diet despite no differences in body weight.

MAP's of the various animal groups are shown in Figure XI.2. Five week old Dahl-S and Dahl-R rats did not differ in MAP. After 8 weeks on the various diets, all rat groups showed significant increases in MAP. On the low salt diet, there was

no difference in MAP between Dahl-S and Dahl-R rats. On the normal and high salt diets, the MAP's of Dahl-S rats were significantly greater than those of Dahl-R rats on the same diet. At the end of 8 weeks on diet, there were no significant differences in MAP's between any of the Dahl-R groups. Thus, MAP in Dahl-R rats was unaffected by salt intake. In Dahl-S rats, the MAP of the high salt group was greater than that of the normal salt group which, in turn, was greater than that of the low salt group.

PHF levels in the various experimental groups are depicted in Figure XI.3. PHF activity was not detectable in Dahl-S or Dahl-R rats at 5 weeks of age. Similarly, PHF activity was never detected in the plasma of Dahl-R rats at the end of the diet study, regardless of salt intake. In Dahl-S rats, PHF activity was not detectable in animals on the low salt diet but was seen in the groups of normal and high salt. Although the absolute PHF level appears higher in the high salt group, statistically, *post hoc* analysis showed no difference in PHF levels between the normal and high salt groups. Within the three Dahl-S diet groups, linear regression analysis showed that PHF level correlated with MAP (Figure XI.4, $r = .50$, $P = .0077$).

D. DISCUSSION

The results of this study show that Dahl-S rats express PHF-like activity on normal and high salt diets and that such PHF-like activity is correlated with the MAP of the animal. Dahl-R rats, on the other hand, do not express PHF activity at any level of salt intake. Although PHF level is correlated with MAP in Dahl-S rats across

the various salt intakes, such a relationship does not necessarily prove a causative role for PHF in the hypertension induced by salt in Dahl-S rats. Such a relationship is suggested, however, by observations in DOCA-treated rats (3) and in SHR rats on low calcium diets (11), where PHF appearance precedes the onset of hypertension. Further evidence of a causative role for PHF in increasing blood pressure is provided by the observation that injection of a polyclonal PHF antibody into SHR can decrease blood pressure (12). Thus, PHF may contribute to the hypertension caused by salt in Dahl-S rats. The fact that PHF levels were similar in Dahl-S rats on normal and high salt diets, but that their MAP's were different, may suggest that PHF is not totally responsible for the entire increase in blood pressure caused by salt. Rather, PHF may have a permissive effect in allowing salt to increase blood pressure. Alternatively, larger sample sizes from each group may have revealed significant differences between the two salt treatments. In addition, although PHF levels are increased in association with a higher salt intake, it is not clear whether salt directly results in PHF expression and whether such an effect is a threshold or dose-dependent phenomenon. Preliminary studies involving adminstration of PHF into normotensive rats have shown that PHF seems to decrease sodium clearance and urine volume (13). Both features are characteristic of Dahl-S compared to Dahl-R rats on high salt diets (14) and of the effect of perfusing normotensive rat kidneys with Dahl-S blood (15). In salt-sensitive human hypertensive patients, PHF levels were directly correlated with the magnitude of blood pressure changes on low and high salt diets (16). Thus, based on these and the current study, PHF expression may

be stimulated by a high salt intake and, in turn, may act on the kidney to promote sodium retention. An intriguing possibility therefore exists, whereby a vicious circle might be established with PHF causing renal salt retention and the salt retention facilitating PHF expression.

Although PHF may induce a salt-sensitive hypertensive state, the induction and maintenance of hypertension in Dahl rats would seem to be multifactorial. In Dahl-R rats, hypertension can be induced by receipt of a kidney transplanted from a Dahl-S rat (17). Similarly, injection of serum from nephrectomized Dahl-S rats does not increase blood pressure in normotensive rats, whereas serum from kidney-intact animals does increase blood pressure (7). Thus the kidney would also seem to play a role in the development of hypertension in Dahl-S rats. Whether the kidney produces a circulating hypertensive factor, activates existing factors, or whether it has a functional abnormality resulting in sodium retention and salt-sensitivity, has not yet been fully established. A similar situation exists in DOCA-salt rats, where both DOCA and salt are necessary to induce PHF expression and hypertension (3). In addition, other factors, such as altered sympathetic activity (18-20) and baroreceptor reflex abnormalities (21-23), may contribute to the hypertension in Dahl-S rats.

In conclusion, the current study shows that PHF-like activity is expressed in Dahl-S rats and that such expression is related to a salt-induced increase in blood pressure. PHF may therefore be (one of) the circulating hypertensive factor(s) hypothesized to exist in Dahl-S rats. The exact role of PHF in salt-sensitivity, and the extent of its contribution to hypertension in Dahl-S rats, remain to be elucidated.

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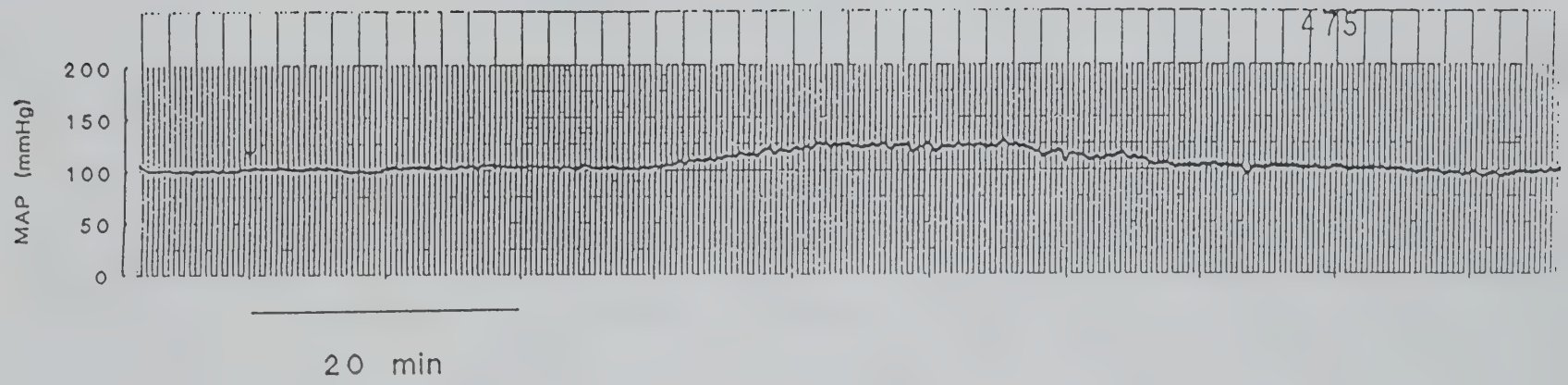

Figure XI.1.

Representative recording shows typical Parathyroid Hypertensive Factor (PHF) -like effect on mean arterial pressure (MAP) in normotensive assay rats. Dialyzed plasma from a Dahl-S rat on a high salt diet was injected at the time indicated (arrow). MAP begins to increase after about 30 minutes, peaks at 50 minutes, and returns to baseline by 70 minutes.

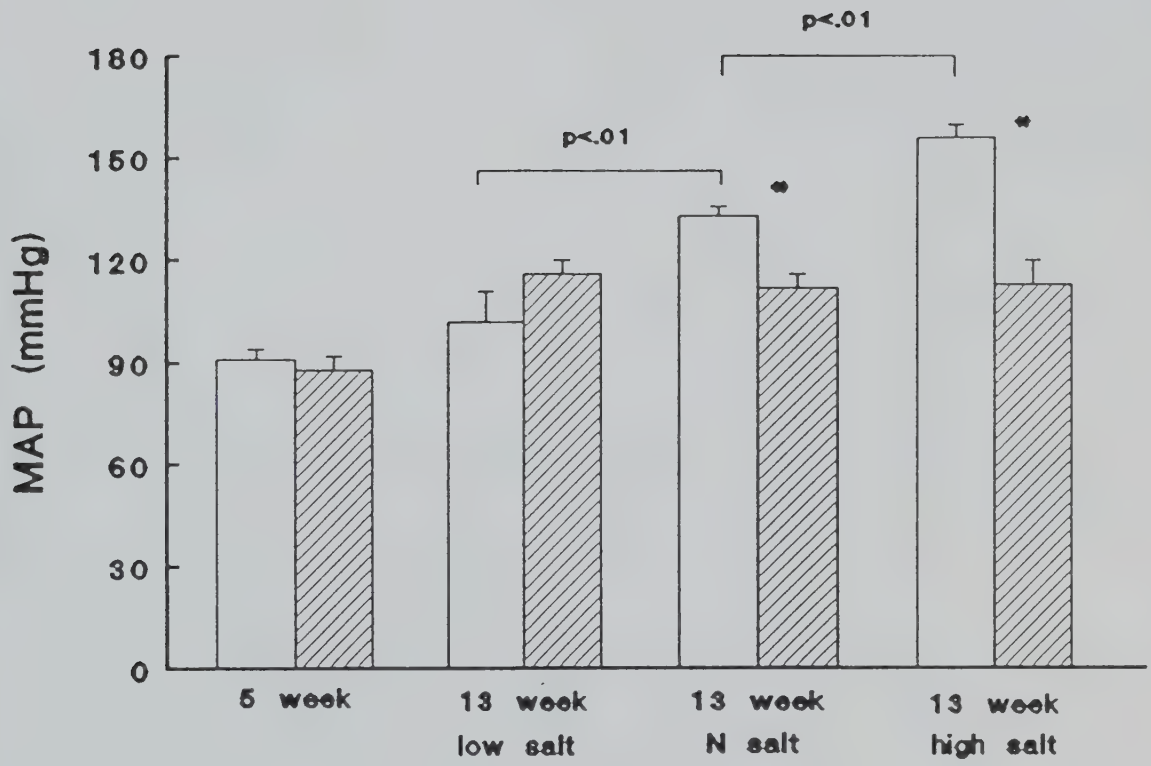

Figure XI.2.

Bar graph shows mean arterial pressures of a representative group of rats at the beginning of the study (5 weeks) and of the various groups at the end of the feeding study (13 weeks). Open bars represent Dahl-S rats; cross-hatched bars represent Dahl-R rats. All 13 week groups had higher mean arterial pressures at 13 weeks than their respective controls at 5 weeks. N salt, normal salt diet. $^{*}P < .005$ Dahl-S vs Dahl-R within same diet group.

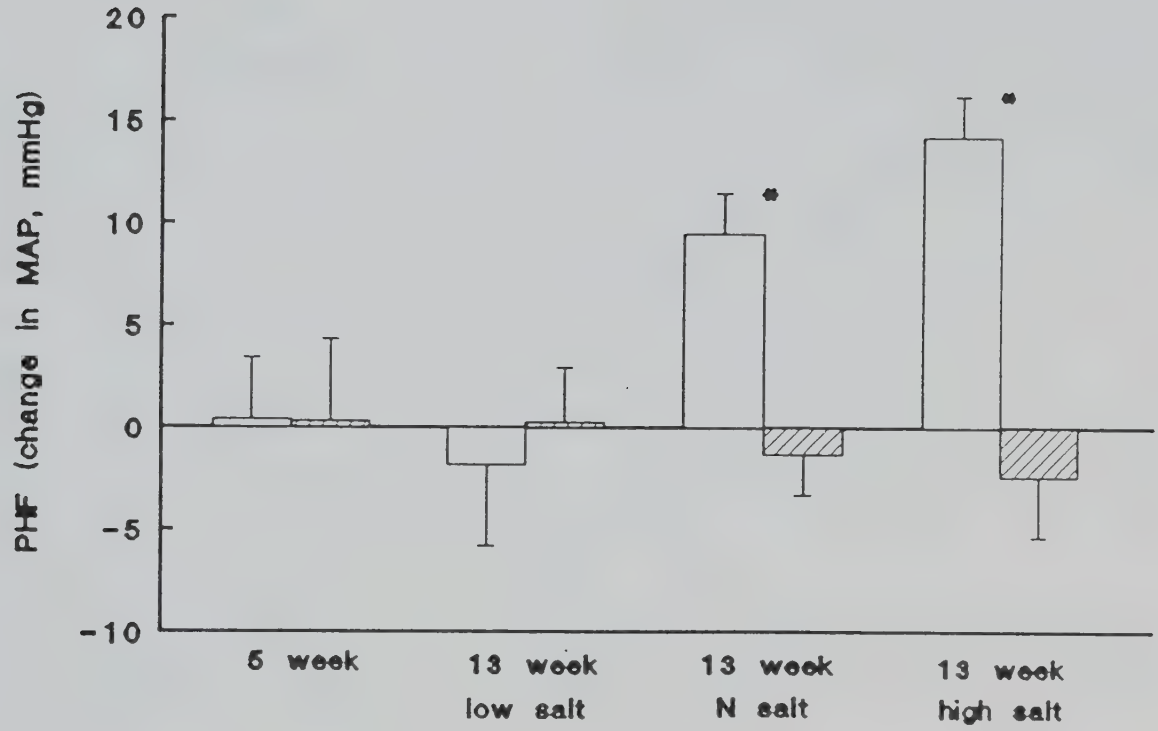

Figure XI.3.

Bar graph shows Parathyroid Hypertensive Factor levels of the various experimental groups. Open bars, Dahl-S rats; cross-hatched bars, Dahl-R rats. N salt, normal salt diet. $^{*}P < .001$ vs 0 based on the null hypothesis that change in mean arterial pressure after injection of test plasma should equal 0.

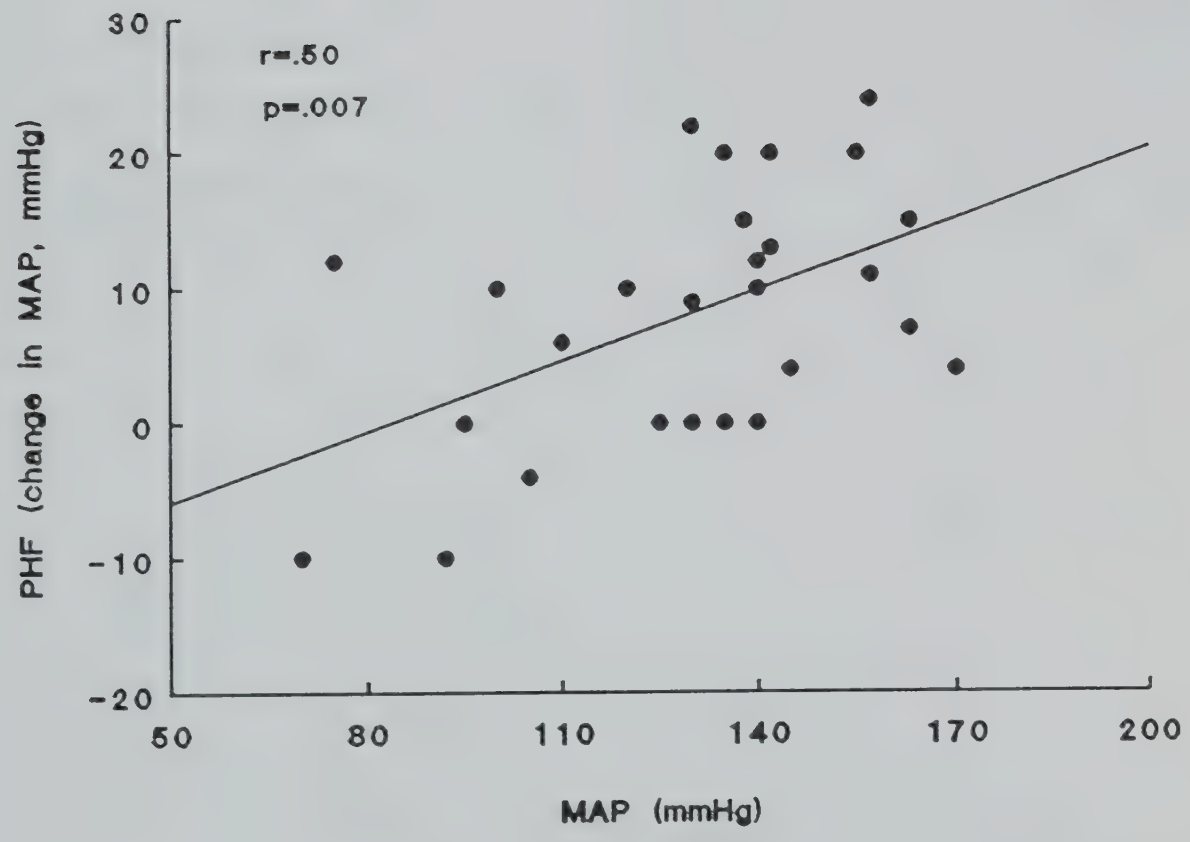

Figure XI.4.

Scatter plot shows relationship between Parathyroid Hypertensive Factor activity and mean arterial pressures in Dahl-S rats at 13 weeks. Results from rats at all levels of salt intake were combined. Solid line represents calculated regression line.

Table XI.1.

Weights of Dahl rats on various salt intakes

	Weights (g)	
	Dahl-S	Dahl-R
5 week Control	139±8	157±3*
13 week Low Salt	182±11†	180±3†
13 week Normal Salt	398±10	401±11
13 week High Salt	377±11	314±7*†

Results are mean ±SEM. *$P < .05$ R vs S. Within 13 week groups: †$P < .01$ vs normal salt group of same strain.

XII. DISCUSSION

Although each set of experiments was discussed in its respective chapter, the following is intended to serve as a summary of the findings and/or their significance. In addition, the following discussion is designed to place these findings in overall context within the field of hypertension.

A. SUMMARY

To summarize the project, the presence of a novel circulating hypertensive factor in spontaneously hypertensive rat (SHR) plasma has been described. This factor is hypothesized to act by increasing intracellular calcium in vascular smooth muscle, as suggested by its effect on calcium uptake in vascular smooth muscle. This substance can also potentiate the actions of other pressors, such as norepinephrine, arginine vasopressin and angiotensin II. A high calcium diet inhibits, and a low calcium diet stimulates, secretion of the factor. Action of the factor is likewise inhibited by calcium channel blockers, again suggesting that its action involves increased calcium entry into vascular smooth muscle. The factor differs from the ouabain-like factors because it stimulates, rather than inhibits, Na^+-K^+ ATPase activity. With respect to the action of parathyroid hormone (PTH), the factor seems to block PTH vascular actions by inhibiting cAMP generation. In SHR, the source of the factor appears to be the parathyroid gland. On this latter basis, the factor was

named "Parathyroid Hypertensive Factor", or "PHF". In humans, PHF-like activity is detected in some hypertensive patients. A possible parathyroid gland source for PHF in humans is suggested by virtue of its occurrence in a proportion of hyperparathyroid patients and its disappearance in such patients post-parathyroidectomy. In animal models, PHF is associated with low renin, salt-sensitive forms of hypertension.

If the above observations are borne out, the existence of PHF has the potential to explain a number of the existing controversies in the field of hypertension. First, PHF may explain the apparent state of mild secondary hyperparathyroidism in low renin forms of hypertension -- both animal and human. This is because PHF, if it interferes with PTH-stimulated cAMP generation in vascular smooth muscle, may also inhibit the actions of PTH in other tissues. As discussed in the Introduction, evidence for such interference is, indeed, present (1-5). Thus, the presence of PHF might more accurately be described as causing a mild state of pseudohypoparathyroidism, as in the case of human pseudohypoparathyroidism type II, wherein a circulating inhibitor blocks PTH action. It is, therefore, of interest to note that pseudohypoparathyroid type II patients are often hypertensive (6).

The existence of PHF, and its isolation, may be one of the first pieces of supporting evidence confirming years of circumstantial reports suggesting the presence of a circulating hypertensive substance(s) and pressor-potentiating factor(s). Indeed, such evidence was first described in the parabiosis experiments of Dahl (7), where hypertension could be induced in a salt-resistant normotensive rat by parabiosis with a salt-sensitive hypertensive rat. It is, therefore, significant to note that

PHF was found in Dahl-S rats in the current study. The parabiosis experiments of Dahl were expanded upon by Zidek *et al.* (8), who also found an increase in blood pressure in Wistar-Kyoto rats (WKY) connected in cross-circulation to SHR. It is of interest in this model to note that the onset of hypertension in the normotensive rats did not occur until after 30 minutes of cross-circulation, raising the question of whether such an effect may have been due to PHF. In a similar manner, the effect of PHF in potentiating other pressor agents may explain the reports from over a decade ago, wherein crude SHR plasma or serum was found to have the same effect (9-12). More recently, plasma from some hypertensive humans and animals has been reported to increase intracellular calcium levels in tissues such as platelets (13) and lymphocytes (14). Again, one can speculate as to whether PHF is involved in such effects. Although studies are currently underway, preliminary analysis of plasma samples from pregnant women for PHF revealed an association between PHF and the development of pre-eclampsia (Sowers and Zemel, personal communication). These same patients with increased PHF levels had also been shown to have increased platelet intracellular calcium levels (15). In a more direct study, Resnick (16) demonstrated the ability of PHF to cause shifts in intracellular magnesium and pH in red blood cells of a magnitude seen in low renin hypertensive patients and which has since been shown to be associated with an increase in intracellular red blood cell calcium.

The relationship of PHF to other described alterations of cellular membrane regulatory processes in hypertension will also be of interest to investigate in future.

Such processes include described alterations in Na^+:H^+ exchange (17), Na^+:Ca^{++} exchange (18), and Ca^{++} ATPase (19).

A third area where the existence of PHF may help resolve controversy is in the role of dietary calcium and hypertension. As described earlier, some hypertensive patients (and animal models) respond favorably to dietary calcium (20,21), whereas others do not (22-24). Again, it appears to be low renin patients -- the ones with lower ionized calcium and higher PTH levels -- who respond best to dietary calcium (25). Other hypertensive patients may show no effect from dietary calcium or, as in the case of two kidney-one clip hypertensive rats (26), may even show an increase in blood pressure in response to dietary calcium. Thus, the presence of PHF seems to also correspond to calcium sensitivity and may identify those hypertensive patients in whom calcium supplementation may be desirable. An analogous situation exists with respect to salt sensitivity. Again, it is the low renin patients and animals who are salt-sensitive. Such patients and animals are also the ones who have PHF. In fact, as part of our clinical studies, baseline PHF correlated with blood pressure response to alterations in salt content of the diet (Resnick, unpublished observations). Thus, PHF may also be a useful marker of salt sensitivity in hypertension. Moreover, PHF, when injected into Sprague-Dawley (SD) rats, has been shown to decrease sodium clearance (27). Thus, PHF may also play a causal role in salt sensitivity. Nevertheless, much work remains to be done in the area of hypertension, renal dynamics and PHF.

A fourth area where PHF may help resolve confusion is in the apparent role of the parathyroid gland, but not parathyroid hormone, in some animal models of

hypertension. As previously described, the parathyroid gland is thought to be implicated in the maintenance of hypertension in some animals by virtue of the fact that parathyroidectomy decreases blood pressure in such animals (28-31). Nevertheless, PTH is consistently hypotensive (32,33). The existence of a second pressor substance of parathyroid gland origin -- namely PHF -- would thus explain this apparent paradox.

Finally, PHF may explain the variable association of hypertension in primary hyperparathyroidism. While some investigators also suggest that PTH may cause the hypertension of hyperparathyroidism, this does not explain why only a portion of hyperparathyroid patients are hypertensive, even though they all have, by definition, increased PTH levels. As shown in Chapter X, hypertensive and normotensive patients could not be separated on the basis of PTH or calcium level. The presence of PHF in many of the hypertensive patients, its disappearance post-operatively, and the fall in blood pressure post-operatively, all suggest a role for PHF in the hypertension of hyperparathyroidism, and may thereby explain why only a proportion of hyperparathyroid patients are hypertensive. Despite the presumed role of PHF in hypertension in hyperparathyroidism, it would still seem that a few patients have hypertension and hyperparathyroidism but do not have PHF. In our series, one patient had these characteristics but did not have a decrease in blood pressure post-operatively. Thus, as described in Chapter X, PHF could be used as a predictor of the effect of parathyroidectomy on blood pressure in hyperparathyroid patients. In the case of hypertensive PHF "negative" hyperparathyroid patients, such patients

seem to have higher renin levels than PHF "positive" hyperparathyroid patients (Resnick, personal communication). The existence of the former class of patients is not inconsistent, however, as one might presume that the high incidence of essential hypertension, together with the relatively high incidence of primary hyperparathyroidism, may result in some patients having both conditions for unrelated reasons. Conversely, it is also interesting to note that essential hypertensive patients are at increased risk for hyperparathyroidism (34). Whether this represents a manifestation of increased parathyroid gland activity is interesting to speculate upon.

B. IS PHF A NEW FACTOR?

In the preceding discussion, PHF has been concluded to be a "new" factor. Nevertheless, many substances with vasoactive properties exist. What, then, is the evidence that PHF is truly a novel substance?

First, PHF has a truly unique time course for blood pressure change, peak effect on calcium uptake, and peak pressor potentiating effect. Despite intensive review of the literature, no other substance appears to have such a delayed action. It is, in fact, probably because of this delayed action that PHF has not been previously described. Nevertheless, certain existing factors deserve to be excluded as candidates for PHF on the basis of either their parathyroid origin or on the basis of their own unique properties.

The first substance to be excluded is endothelin. This substance, first described in 1988 (35), is an extremely potent vasoconstrictor. Its vascular actions are unique

in that they are tripartite. Following intravenous injection, an initial rapid, transient increase of blood pressure occurs, followed by a somewhat sustained (about 5 minute) depressor response. Following this is a third phase of prolonged hypertension of lesser magnitude than the first phase. This third phase ends about 30 minutes post-injection. Nevertheless, the blood pressure profile of endothelin is different from PHF in two ways. First, it has three phases, compared to the one phase of PHF action. Secondly, although its action is prolonged, the onset and peak effect still occur earlier than those of PHF. More compelling, however, is the fact that our PHF antibody shows no cross-reactivity with endothelin (Tang, Benishin and Pang, unpublished observations).

Another set of substances to be considered as PHF candidates are fragments of PTH. Hesch *et al.* (36) have suggested that N-terminal-deleted fragments of PTH may have hypertensive properties. Nevertheless, such a claim has not been substantiated, nor have other investigators shown vascular actions of any PTH fragments other than those containing the 1-34 sequence (37). In Chapter VIII, Figure VIII.1 shows an infusion of PTH, bPTH-(3-34) and PHF. bPTH-(3-34) can be seen to have no pressor effect, despite prolonged infusion and despite the fact that a PHF effect is clearly evident by this time in animals infused with PHF. In addition, as with endothelin, the PHF antibody shows no cross-reactivity with numerously tested fragments of bovine, rat or human PTH, including the intact 1-84 PTH (Tang, Benishin and Pang, unpublished observations).

Also necessary to exclude as PHF candidates, by virtue of parathyroid

secretion, are PTH-related peptide (PTHrp) (38) and chromogranin A (39). Of particular note is that PTHrp has approximately a ten-fold more potent vascular depressor effect than PTH (Pang and Shan, unpublished observations). Chromogranin A, as well, is known to be hypotensive (39), and neither chromogranin A nor PTHrp reacts with the PHF antibody (Tang, Benishin and Pang, unpublished observations). Thus, neither chromogranin A nor PTHrp appear to be suitable candidates for PHF. PHF, therefore, appears to truly be a unique substance of parathyroid gland origin.

C. ARE THE ACTIONS ATTRIBUTED TO PHF DUE TO A SINGLE SUBSTANCE?

PHF activity was originally described as being contained in certain plasmas or serums. By virtue of the temporal relationship between the blood pressure effect, the effect on calcium uptake and the effect on pressor potentiation, PHF appears to be due to one substance. Plasma and serum, however, contain numerous substances which may all have differing vascular and calcemic effects. It was thus necessary to show that the characteristic actions of PHF were due to a single substance.

Through the efforts of Dr. C. Benishin, PHF has been purified to homogeneity (40) and the existence of a single species has been confirmed by mass spectroscopy. When this purified PHF is tested in the bioassay system, characteristic PHF activity is seen. In addition, semipure PHF has been confirmed to increase free intracellular calcium levels in vascular smooth muscle as measured by Fura-2, with a time course consistent with calcium uptake and pressor potentiating effects (41). Characteristic

PHF-like activity, therefore, can be ascribed to one substance.

D. THE NATURE OF PHF

Because of its unique vascular effects, the nature and mechanism of action of PHF are of great interest. With respect to the nature of PHF, structure determination is currently underway. With respect to its mechanism of action, this too is currently under investigation. Although PHF inhibits the cAMP stimulatory effect of PTH, as described earlier, this is almost certainly not the way in which PHF affects blood pressure, calcium uptake and other physiological parameters. Because of the length of time necessary to see a PHF effect, it might be speculated that a delayed process, such as protein synthesis, might be necessary for PHF effect. Such a delayed second messenger process is not unique in vascular tissue. Webb described that phorbol esters (stimulating protein kinase C directly) take up to 1 hour to have a vascular effect *in vitro* (42). PHF, however, does not seem to stimulate the PLC pathway (44). With regard to protein synthesis, the use of an *in vivo* bioassay system makes the use of protein synthesis inhibitors difficult. A steroid effect might also be hypothesized, but PHF's large size (approximately 3,000 molecular weight) in relation to steroid molecules also makes this unlikely. The mechanism of action of PHF, therefore, remains to be elucidated. Quite possibly its end effects may be due to a number of processes.

E. THE PARATHYROID ORIGIN OF PHF

Studies included in this work suggest that PHF has a parathyroid origin for a number of reasons. First, factors that affect parathyroid gland function, such as changes in dietary calcium, also affect PHF. Secondly, parathyroidectomy in SHR results in disappearance of the factor from the plasma and a fall in blood pressure of the parathyroidectomized animal (Chapter VIII). Third, in cross-transplantation experiments, hypertensive and PHF activity in the plasma tracked with the parathyroid gland, not with the animal (Chapter VIII). Fourth, in human hyperparathyroid patients, only hypertensive patients had PHF-like activity in their plasma, and such activity disappeared post-parathyroidectomy, together with an associated fall in blood pressure (Chapter X). Finally, in SHR, a novel cell type was observed in the parathyroid gland (43), numbers of which correlated both with blood pressure and PHF level. All of this evidence suggests a parathyroid gland origin of PHF. Nevertheless, all such evidence is circumstantial -- direct evidence is needed for proof. Such evidence has now been obtained.

Direct evidence for the parathyroid gland origin of PHF has come from three sources. First, culture of parathyroid glands results in the appearance of PHF-like activity in the culture media from SHR, but not WKY, parathyroid glands (44). Second, a peak with PHF-like activity can be purified to homogeneity from SHR parathyroid gland culture media, and such a peak has an identical elution profile during HPLC purification to plasma-purified PHF (44). Finally, antibodies raised to PHF isolated from culture media cross-react with PHF purified from plasma (45). In

SHR, therefore, PHF definitely appears to be of parathyroid gland origin. Because of its disappearance from the plasma after parathyroidectomy, the parathyroid gland also would seem to be the major source of PHF production in SHR. Similar studies are currently underway using culture material from human parathyroid gland adenomas.

F. THE ROLE OF PHF

The functional significance of PHF is still somewhat unclear. Certainly, it is closely associated with low renin forms of hypertension, but whether it is present at low levels in normal persons, or whether it is only expressed in certain pathological conditions, remains to be established. Such a determination will require a more sensitive immunological assay than the current, rather crude, bioassay.

One of the major questions regarding PHF is whether it plays a causative role in hypertension, whether its expression is secondary to the hypertension, or whether its expression is an epiphenomena of some other underlying cause for hypertension. The question of whether it is expressed as a result of hypertension is relatively straightforward. If PHF expression were due to increased blood pressure *per se*, it should be found in all forms of hypertension. From Chapter XI, it can be seen that, given the same rat strain, PHF becomes expressed in the low renin form of hypertension (DOCA-salt), but not the high renin form (2K-1C), despite similar increases in blood pressure. Similarly, in Chapters VII and XII, PHF levels are seen to be significantly increased in some cases prior to any significant increase in blood pressure.

Thus, PHF expression is not secondary to hypertension. In the latter two cases, its presence prior to the onset of hypertension argues for a causative role in hypertension. Similarly, its *in vitro* effects on intracellular calcium would also be expected, *in vivo*, to increase blood pressure. Further evidence for a causative role of PHF in hypertension has been obtained from antibody studies. Injection of a small amount of PHF antibody into an SHR rat reproducibly and dose-dependently decreases blood pressure (45). This latter observation is, at present, the best evidence for a causative role of PHF in hypertension. Other evidence suggestive of a causative role for PHF in hypertension comes from observations that any factor which influences PHF levels, such as changes in dietary calcium or salt, also influences blood pressure. Although such relationships alone may be explained as an epiphenomenon, certainly the demonstration of (1) a direct hypertensive effect, (2) interventions which alter PHF levels alter blood pressure (and *vice versa*), and (3) the hypotensive effects of PHF antibodies *in vivo*, provide as good evidence as is currently obtainable for the causative role of any substance in hypertension. For example, in the case of renin, which is well accepted as playing a role in some forms of hypertension, evidence for its causative role relies on (1) the demonstration that renin, itself, can lead to a pressor effect, (2) that factors which affect renin can affect blood pressure in affected individuals, and (3) inhibitors of renin action can reduce blood pressure. These criteria are also apparently fulfilled in the case of PHF, although instead of a pharmacological inhibitor of action (such as saralasin for renin), an antibody was used. To further ensure specificity of antibody action, preincubation of SHR plasma

with anti-PHF antibody also removes the hypertensive effect of the plasma (Benishin, unpublished observation). There is, therefore, strong evidence that PHF may play a causative role in some forms of hypertension.

Despite an apparent causative role for PHF in some forms of hypertension, it has been observed that some normotensive humans also have PHF-like activity in their plasma (Chapter IX). This, at first, may seem inconsistent with a causative role for PHF in hypertension, but, with closer examination, is not. Using other hypertensive substances as a precedent, circumstances exist when normotensive patients can have high levels of pressor substances, yet be normotensive. Two examples are high renin levels with dehydration, or high aldosterone levels when on a low salt diet. By extrapolation, however, this would imply a possible physiological role for PHF. This may also be possible, based on some preliminary data. First, in normotensive humans, if one separates by age and race, PHF correlates inversely with renin profile such that the rank order of mean PHF levels is highest in black females, followed by black males, then white males and white females. Plasma renin activity has a completely reversed rank order in these individuals (Resnick, personal communication). These data are based on assays of over one hundred normotensive patients, but confirmation will require, as mentioned, a more specific assay.

A second explanation for the presence of PHF in normotensive individuals also stemmed from the assay of PHF in the above group. That is, PHF may be indicative of a prehypertensive state. Indeed, normotensive patients with elevated PHF levels also showed some metabolic abnormalities characteristic of low renin hyper-

tension, such as increased urinary calcium excretion (46). Such PHF "positive" normotensive patients are currently being followed to see if hypertension will develop.

Although determination of whether PHF is normally expressed at low levels in individuals will require a more sensitive assay, it would seem that normotensive individuals can be induced to express PHF under certain circumstances. First, in normotensive WKY rats, PHF and hypertension can be induced by DOCA-salt. Similarly, although we were unable, a Russian group claims the induction of PHF in WKY rats by exposure to a low calcium diet (Tchurina *et al.*, personal communication). In humans, as well, PHF has been found gestationally in some pre-eclamptic women and disappears post-partum (Sowers and Zemel, personal communication). Thus, it is possible that PHF may be expressible in otherwise normal humans, or that it may be present constitutively at low levels and increased under specific pathological conditions.

Presuming PHF is present constitutively in humans, what is its role? This is entirely speculative, but one possibility may be in maintaining blood pressure. Such a function is possibly suggested in patients with shock, who often have increased PTH and decreased ionized calcium levels (47), just as their low renin hypertensive counterparts have. Is such a pattern of low intracellular calcium and increased PTH indicative of PHF presence? We are unsure, but do intend to study such patients. Further to a possible role in blood pressure regulation, if PTHrp is important in blood pressure control, and if PHF blocks the vascular actions of PTH, PHF might

also be expected to block the vasodilating action of PTHrp. Thus, PHF and PTHrp may represent a "Yin and Yang" situation, much as endothelin and EDRF seem to function as paracrine controllers of microcirculation. This supposition is also purely speculative and will require considerable experimental investigation.

Another possible role for PHF is in maintaining intracellular calcium levels. Generally, calcium homeostasis is considered to involve a relationship between calcium absorption from the gut, calcium release from bone, and renal calcium loss. However, calcium is also exchanged across cell membranes, meaning that such exchange would also need to be considered in total plasma calcium homeostasis. If calcium regulating hormones exist to regulate gut, renal and bone homeostasis, is it not reasonable to presume that a hormone to regulate intracellular calcium might also be needed? This too, however, is purely speculative and would also require experimental proof.

G. CLINICAL IMPLICATIONS OF PHF

Although the clinical implications of PHF may not yet be fully established or appreciated, several possibilities are even now evident.

First, if PHF is a causative factor in low renin hypertension, its discovery would represent a significant advance in the field of hypertension. Currently, 10% or less of the causes of hypertension are known. With the discovery of PHF, up to an additional 40% of cases might be explained. This is probably being optimistic, as even within the low renin group, multiple causative factors are probably operational.

Nevertheless, even if PHF is only contributing to low renin hypertension, it still represents an advance in the understanding of hypertension. And, ultimately, one cannot consider a cure until a cause is known.

More immediate use may possibly be made of PHF findings, however. First, and of great clinical utility, would be the measurement of PHF as a method of identifying salt-sensitive and low renin hypertensive patients. In the former case, only about 50% of hypertensive patients show a decrease in blood pressure with salt restriction (salt-sensitive), whereas 15% of patients may actually have an increase in blood pressure with salt restriction. Thus, to prescribe treatment to those in whom it will be effective, a simple method of identifying such individuals would be of use. Currently, renin profiles can be used, but this has the disadvantages of requiring a 24 hour urine specimen and a technically fastidious radioimmunoassay. PHF measurements, based on preliminary results, would require only a single blood test. In the case of calcium supplementation, a similar situation would exist.

With respect to renin status, this is often used as an indication of most appropriate pharmacotherapy, with low renin patients usually being more responsive to calcium channel blockers and diuretics. Again, the complicated nature of renin profiling precludes its widespread clinical use. PHF measurement, when an immunoassay becomes available, may then allow the practitioner to select the therapy most likely to be effective -- both pharmacologic and non-pharmacologic -- without the need for "trial and error". Similarly, if a recent report linking status to prognosis in hypertension is true (48), PHF measurement may also be potentially useful to determine

prognosis, with high PHF patients presumably having a better prognosis than those with PHF-independent hypertension. Finally, in hypertensive patients, PHF measurement may indicate the effect of parathyroidectomy on blood pressure, such that it may be an additional factor for or against surgery.

H. FUTURE DIRECTIONS

The current study, and associated studies on PHF alluded to herein, probably pose as many questions as they have answered. Particularly important areas for future study will include the determination of the structure and mechanism of action of PHF. In addition, further work will be necessary to develop a readily available immunoassay for PHF which will allow for epidemiological studies. Similarly, a more sensitive assay will also allow dynamic studies of factors which influence PHF to be carried out. Of course, the genetic source and control of PHF will be important to fully understand its actions, expression and epidemiology. Finally, as alluded to above, the possibility of drugs specifically targeted against PHF may have utility in future. Hopefully, this work is but an introduction to a new field in hypertension research.

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